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Preliminary communication

Synthesis of some novel benzoxazole derivatives as anticancer, anti-HIV-1 and antimicrobial agents☆

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Abstract

In an effort to establish new candidates with improved antineoplastic, anti-HIV-1 and antimicrobial activities we report here the synthesis and in vitro biological evaluation of various series of 2-substituted benzoxazoles: 2-[(Arylhydrazono, arylidene, cycloalkylidene and N-substituted thiocarbamoyl)cyanomethyl]-benzoxazoles(**2–4** and **7**, respectively); 2-[(4- or 5-oxothiazoliden-2-yliden)benzoxazoles (**5** and **6**) and 2-(4-amino-3-substituted-2-thioxo-2,3-dihydrothiazol-5-yl)benzoxazoles (**8**), together with the synthesis of some substituted 3*H*-pyrido[2,1-*b*]benzoxazoles (**9–11**). The absolute configuration of compound **3b** was determined by X-ray crystallography. The results of the in vitro anticancer screening revealed that some of the tested compounds exhibited broad spectrum antitumor activity. The most active compounds are **2a**, **3b**, **8a** and **8d**, their GI_{50} MG-MID values: 37.7, 19.1, 20.0 and 15.8 μM; TGI MG-MID values: 75.9, 53.7, 53.7, and 58.9 μM; and LC_{50} MG-MID values: 97.7, 93.3, 89.1and 93.3 μM, respectively. The in vitro microbiological data showed that compound **7c** was the most active against *Staphylococcus aureus* (minimal inhibitory concentration (MIC) < 12.5 μg ml^{-1}). While compounds **5**, **8a**, and **8d** were the most active against *Bacillus subtilis* (MIC values < 12.5 μg ml^{-1}). On the other hand, compounds **5** and **7c** were the most active against *Escherichia coli* (MIC < 25 μg ml^{-1}), their activity is about half the activity of ampicillin and streptomycin . In addition, compound **4b** and **7c** were the most active against *Pseudomonas aeruginosa* (MIC < 25, 50 μg ml^{-1}). Compound **4b** was two times as active as ampicillin and streptomycin while compound **7c** was active as both. The results of antimycotic activity indicated that, Compound **7c** showed mild activity against *Candida albicans* when compared with clotrimazole (MIC < 100 μg ml^{-1}). In vitro HIV-1 testing revealed that compound **7a** displayed moderate anti-HIV-1 activity (maximum % cell protection, 36.6 at 2×10^{-5} μM).

Keywords: 2-Cynomethylbenzoxazole; 2-Arylidenecynomethylbenzoxazoles; 2-Hydrazonocynomethylbenzoxazoles; 2-(2,3-Dihydrothiazol-5-yl)benzoxazoles; 2-Thiocarbamoylcyanomethylbenzoxazoles; 3*H*-pyrido[2,1-b]benzoxazoles; Anticancer; Anti-HIV-1; Antimicrobial activity

1. Introduction

In the last few years various 2-substituted benzoxazole derivatives were studied extensively for their antitumor [1–7], antiviral [8–14] and antimicrobial activities [15–23] as non-nucleoside topoisomerase 1 poison, HIV-1 reverse transcriptase and/or DNA gyrase inhibitors [12–14]. For example the antibiotic Calcimycin that includes a 2-substituted benzoxazole ring in its molecular structure is very active against *Bacillus cereus*, *Bacillus megaterium*, and *Micrococcus lutes* [15]. The benzoxazole derivative, 3-(4,7-dichlorobenzoxazol-2-ylmethylamino)-5-ethyl-6-methyl-pyridin-2-(1*H*)-one (**L-697.661**, Fig. 1) was found to be an effective non-nucleoside selective HIV-1 reverse transcriptase inhibitor. A combined therapy with zidovudine and **L-697.661** achieved marked decrease of viremia in some primary HIV-infected patients [12–14].

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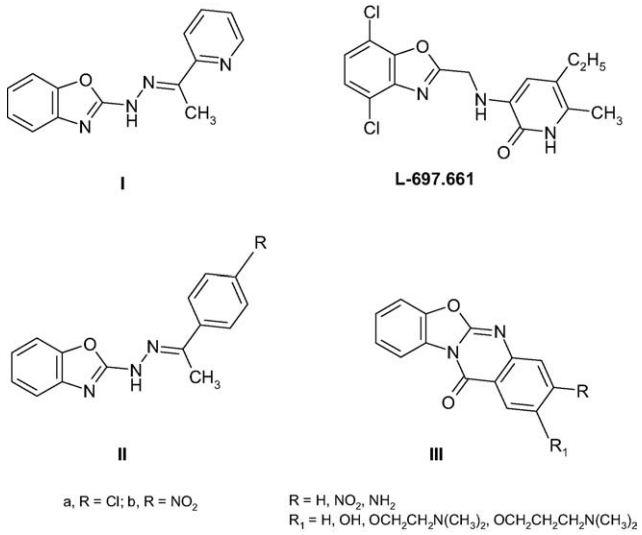


Fig. 1.

Careful literature survey revealed that many hydrazone derivatives are reported as anticancer and antimicrobial agents. For example, 2-[1-(pyridin-2-yl)ethylidene]-hydrazinobenzoxazole (**I** Fig. 1) [5] and 2-[(4-substituted methylbenzylidene)-hydrazino]benzoxazoles (**II** Fig. 1) [19]. Furthermore, the cyano function is included in the structure of some antibiotics like cephacetrile [24], cefmetazole [25] and toyocamycin [26]. In addition, the third and fourth generations of cephalosporins as cefixime and cefepime incorporate thiazole ring in their side chains [27].

In view of the above mentioned findings and as continuation of our effort to identify new candidates that may be of value in designing new, potent, selective and less toxic anticancer, antiviral and/or antimicrobial agents we report in the present work the synthesis of some related new 2-substituted benzoxazoles that comprise the aforementioned moieties in their framework in order to investigate their in vitro antitumor, anti-HIV and antimicrobial activities.

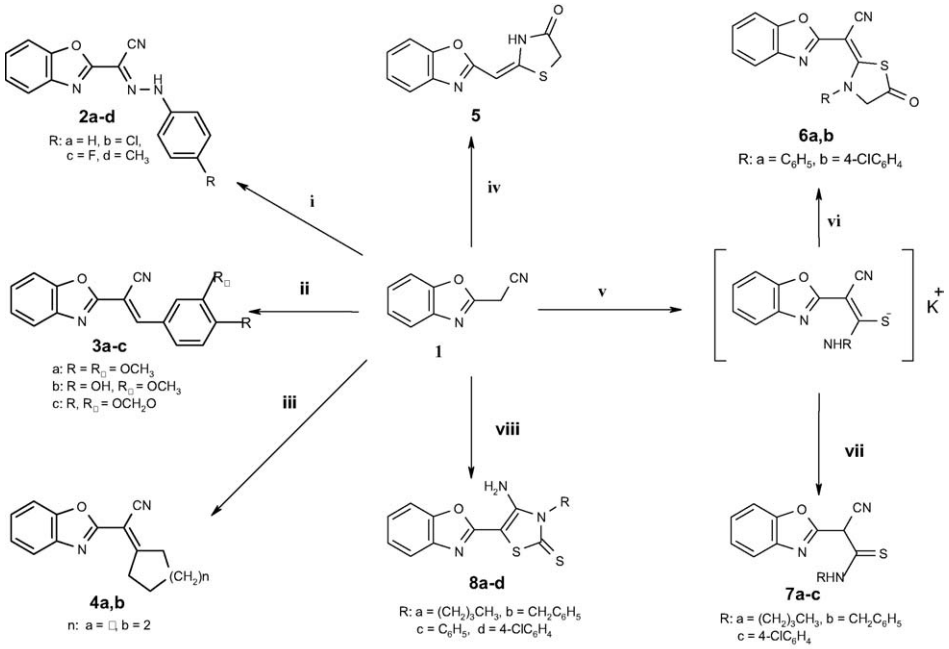
The synthesized compounds include phenyl or substituted phenyl, cycloalkyl, 4- or 5-oxathiazolidene groups linked to benzoxazole through three, two or one atom spacer (compounds **2a–d**, **3a–c**, **4a**, **b**, **5** and **6a**, **b** Scheme 1). These compounds are considered as related structural analogs of the previously reported **I**, **L-697.661** and **II** (Fig. 1).

The synthesis of 2-[(N-substituted thiocarbamoyl)cyanomethyl]benzoxazoles **7a–c** were suggested to investigate the replacement of the phenyl ring or the heterocyclic ring by an open chain containing a thiocarbamoyl moiety. In addition, 2-[(4-amino-3-substituted-2-thioxo-2,3-dihydrothiazol-2-yl]benzoxazoles **8a–d** were designed in which the dihydrothiazole ring is directly attached to benzoxazole.

Moreover, some polycyclic fused benzoxazole derivatives have been reported as potent anticancer such as the tetracyclic 2,3-disubstituted-12*H*-benzoxazolo[2,3-*b*]quinazolin-12-ones (**III**, Fig. 1) [3]. This finding together with the fact that, the majority of DNA intercalating antitumor agents comprising a planer tricyclic and tetracyclic chromophore motivated the design of new series of substituted 3*H*-pyrido[2,1-*b*]benzoxazoles (**9a**, **b**; **10** and **11a–c** Scheme 2) were designed as another molecular variant of **III** (Fig. 1).

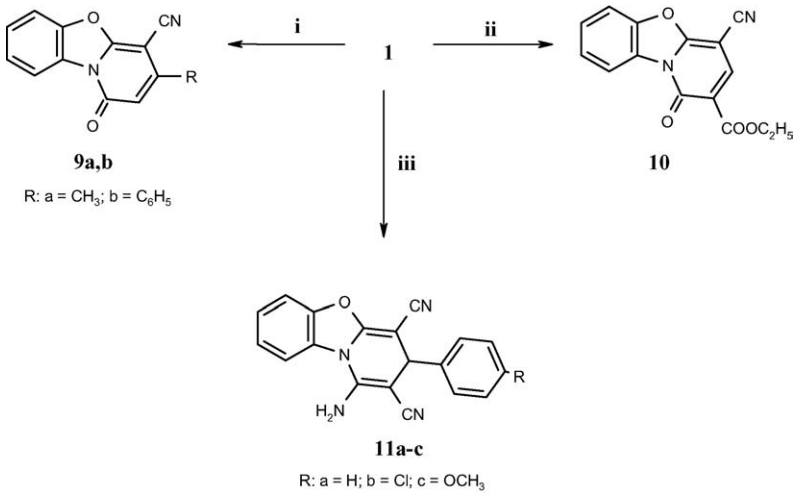
2. Chemistry

The target compounds were synthesized as outlined in Schemes 1 and 2. Diazocoupling of 2-cyanomethyl-



Reagents: i = 4-$RC_6H_4N_2^+Cl^-$□ii = 3-R, 4-$R_□C_6H_3CHO$□iii = c□cl□□□n□an□n□, c□cl□□□□an□n□□i□= $HSCH_2COOH$
□= KOH□RNCS□□i = $ClCH_2COCl$□□ii = HCl□□iii = S□RNCS,□□□□

Scheme 1.



Scheme 2.

benzoxazole **1** with the appropriate diazonium acetate in acetic acid solution afforded the corresponding 2-[(arylhydrazono)-cyanomethyl]benzoxazoles **2a**–**d**. 2-[(Arylidene)cyanomethyl]-benzoxazoles **3a**–**c** were obtained by condensing **1** with the appropriate aromatic aldehyde in ethanol using a catalytic amount of triethylamine. In analogy 2-[(cycloalkylidene)-cyanomethyl]-benzoxazoles **4a**, **b** were prepared by reacting **1** with the selected cyclic ketone in ethanol using ammonium acetate as catalyst.

Preparation of 2-[(4-oxothiazolidene)cyanomethy]-benzoxazole **5** was accomplished by the reaction of 1 with thioglycollic acid through modification of procedure previously reported for the synthesis of analogous compounds [28,29]. On the other hand, the synthesis of 2-[(3-aryl-5-oxothiazoliden-2-ylidene)]bezoxazoles **6a**, **b** and 2-[(N-substituted thiocarbamoyl)cyanomethyl]benzoxazoles **7a**–**c** was achieved as previously described for the preparation of analogous compounds [30]. Accordingly treatment of **1** with the appropriate arylisothiocyanate in dimethylformamide in presence of an equivalent amount of potassium hydroxide produced the potassium salts of 2-[(N-substituted thiocarbamoyl)cyanometyl]benzoxazoles. Cyclization of the latter with chloroacetylchloride in dry dimethylformamide gave 2-[(3-aryl-5-oxothiazoliden-2-ylidene)]bezoxazoles **6a**, **b**. Whereas, acidification of such potassium salts yielded the corresponding benzoxazoles **7a**–**c**. The 2-(3-substituted 4-amino-2-thioxo-2,3-dihydrothiazol-5-yl)benzoxazoles **8a**–**d** were obtained by reacting **1** with sulfur and the appropriate isothiocyanate following the reaction conditions reported for the preparation of related compounds [31].

On the other hand 3-substituted 1-oxo-1*H*-pyrido[2,1-*b*]-benzoxazol-4-carbonitriles **9a**, **b** and ethyl 4-cyano-1-oxo-1*H*-pyrido[2,1-*b*]benzoxazole-2-carboxylate **10** were conveniently synthesized by adapting the previously published procedure for the synthesis of analogous compounds [32,33]. Accordingly, refluxing **1** with an equivalent amount of ethyl aceto or benzoylacetate or diethyl ethoxymethylenemalonate in ethanol resulted in the formation of **9a**, **b** and **10**, respectively. Finally, 1-amino-3-aryl-3*H*-prido[2,1-*b*]benzoxazoles **11a**–**c** were obtained by cyclocondensation of **1** with arylidenemalononitrile applying the previously reported reaction conditions for the preparation of related compounds [34,35]. ^{1}H-NMR spectra of **11a**, **b** characterized by a singlet at 4.61–4.73 ppm due to pyridobenzoxazole-C_3–H, and a D_2O-exchangeable singlet at 6.48–6.57 ppm corresponding to NH_2 indicating the existence of these compounds in the amino rather than imino form.

The structures of the compounds illustrated in Schemes 1 and 2 were confirmed by microanalyses, IR, ^{1}H-NMR and mass spectral data (see Section 4). IR and ^{1}H-NMR spectra of compounds **2a**–**d** revealed the existence of two possible tautomeric forms, –C(CN)=NH–Ar and=C(CN)–N=N–Ar. The IR spectra showed NH stretching absorption and the ^{1}H-NMR (DMSO-d_6) of compounds **2a**, **d** showed two NH signals at different chemical shifts each is integrated for half proton. The X-ray crystallographic analysis of compound **3b** was performed (Fig. 2) as it encountered dual anticancer and antimicrobial activities. It verified that the flat benzoxazole and benzene moieties are non-coplanar to each other. The cyano group and the benzene ring are cited in the cis configuration. On the other hand, the cyano group and benzoxazole nitrogen are in the cisoid conformation. This would add an important dimension for predicting its mode of orientation on the receptor.

3. Biological results and discussion

3.1. Anticancer screening

Fourteen compounds (**2a**, **b**; **3a**–**c**; **4a**, **b**; **8a**–**d**; **9a**, **b** and **10**) were selected by National Cancer Institute (NCI) in vitro

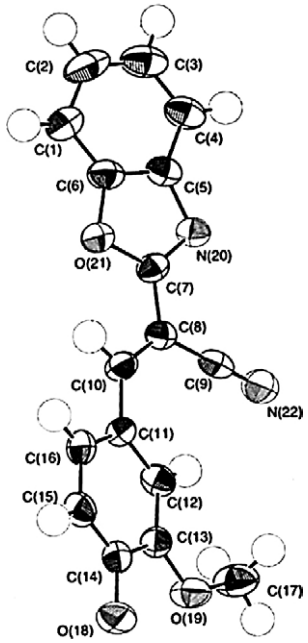


Fig. 2. Structure and solid state configuration of compound **3b**.

disease-oriented human cells screening panel assay [36] to investigate their antitumor activity. About 60 human cell lines derived from nine clinically isolated cancer types (Leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer), were incubated with five concentrations (0.01–100 μM) for each compound and were used to create log concentration–% growth inhibition curves. Three response parameters (GI_{50}, TGI and LC_{50}) were calculated for each cell line. The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compounds causing 50% decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compound resulting in total growth inhibition and the LC_{50} value (cytotoxic activity) is the concentration of the compounds causing net 50% loss of initial cells at the end of the incubation period (48 h). Subpanel and full panel mean-graph mid-point values (MG-MID) for certain agents are the average of individual real and default GI_{50}, TGI, or LC_{50} values, respectively.

In the present study only six compounds namely; 2-[(phenyl or 4-chlorophenylhydrazono)-cyanomethyl]-benzoxazoles **2a**, **b**; 2-[(3-hydroxy-4-methoxybenzylidene)-cyanomethyl]-benzoxazole **3b**, 2-[(cyclohexylidene)-cyanomethyl]-benzoxazole **4b** and 2-[4-amino-3-butyl (or 4-chlorophenyl)-2-thioxo-2,3-dihydrothiazol-5-yl]benzoxazoles **8a**, **b** exhibited potential antitumor activities against most of the tested subpanel tumor cell lines (GI50, TGI and LC50 values < 100 μM Tables 1 and 2).

With regard to the sensitivity against some individual cell lines, Compounds **2a** and **8d** were proved to be effective against CNS cancer (GI_{50} values 21.5 and 12.9 μM, respectively). Compound **2b** was active against leukemia (GI_{50} value 25 μM). Compound **3b** was the most active against ovarian cell line (GI_{50}, 17.3 μM). Compound **4b** showed moderate activity against colon cell line (GI_{50}, 44.7 μM). While compound **8a** proved to be effective against prostate cell line (GI_{50}, 17.9 μM). On the other hand, Compound **8d** showed high

Table 1
Median growth inhibitory concentrations (GI_{50}, μM) of in vitro subpanel tumor cell lines and GI_{50} (μM) full panel mean-graph mid-points (MG-MID)

Compound numbers	Subpanel tumor cell lines[a]									Full panel GI_{50} MG-MID[b]
	I	II	III	IV	V	VI	VII	VIII	IX	
2a	48.3	39.0	58.7	21.5	64.4	26.2	42.1	28.1	43.8	34.7
2b	25.0	58.5	66.9	31.6	69.1	57.8	67.3	88.9	65.0	46.8
3b	25.8	17.6	18.8	18.8	17.7	17.3	25.2	23.5	18.5	19.1
4b	53.0	62.6	44.7	69.0	84.4	73.3	88.9	78.5	59.7	61.7
8a	39.6	20.6	21.9	19.3	22.5	18.2	21.3	17.9	20.1	20.0
8d	72.5	15.9	19.4	12.9	13.6	19.7	25.0	16.9	16.1	15.8

[a] I, Leukemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer.

[b] GI_{50} (μM) full panel mean-graph mid-point (MG-MID) = the average sensitivity of all cell lines toward the test agent.

Table 2
Median total growth inhibitory concentrations (TGI, μM) of in vitro subpanel tumor cell lines, TGI (μM) full panel mean-graph mid-points (MG-MID) and LC_{50} (μM) full panel mean-graph mid-points (MG-MID)

Compound numbers	Subpanel tumor cell lines[a]									Full panel TGI MG-MID[b] (Full panel LC_{50} MG-MID)[c]
	I	II	III	IV	V	VI	VII	VIII	IX	
2a	100	81.1	87.7	56.7	95.8	70.2	77.7	86.2	71.8	75.9 (97.7)
2b	82.6	94.4	100	84.8	98.4	91.2	100	100	99.5	93.3 (100)
3b	86.5	47.6	39.6	50.4	56.2	44.6	69.4	65.8	66.4	53.7 (93.3)
4b	91.1	94.4	89.0	92.9	100	100	100	100	98.6	95.5 (100)
8a	94.6	53.8	75.0	51.9	56.8	38.3	53.6	68.0	61.2	53.7 (89.1)
8d	100	63.7	88.2	32.4	60.3	46.6	70.9	57.5	74.3	58.9 (93.3)

[a] I, Leukemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer.

[b] TGI (μM) full panel mean-graph mid-point (MG-MID) = The average sensitivity of all cell lines toward the test agent.

[c] LC_{50} (μM) full panel mean-graph mid-point (MG-MID) are shown in parentheses.

sensitivity against leukemia K-562 and melanoma UACC-62 at GI_{50} values of 0.47 and 0.45 μM, respectively. Over all, the cancer cell cytotoxicity of the tested compounds is not high.

The ratio obtained by dividing the compound's full panel MG-MID (μM) by its individual subpanel MG-MID (μM) has been considered as a measure of compound selectivity. Ratio between 3 and 6 refer to moderate selectivity, ratios greater than 6 indicate high selectivity towards the corresponding cell line, while compounds meeting neither of these criteria are rated non-selective [37]. All the tested compounds were non-selective with broad spectrum of activity against the nine tumor subpanels tested with ratios ranging between 0.22 and 1.87 (Table 3).

The activity of the tested compounds could be correlated to the structure variations and modifications. Out of the 2-[(arylhydrazono)cyanomethyl]benzoxazoles (**2a–d**), the unsubstituted phenyl hydrazono derivative **2a** was the most active one. However, the activity of **2a** is > 10-fold less than that of the lead compound **II** (Fig. 1). This revealed that replacement of pyridine ring system decreased the corresponding biologic activity. In addition, replacement of hydrogen atom at the 4-position of phenyl group with chlorine (**2b**) resulted in a further decrease in activity towards all tumor subpanel cell lines except leukemia.

In case of 2-[(arylidene)cyanomethyl]benzoxazoles **3a–c**, it was concluded that substitution at position 3- and 4- of the phenyl group by two methoxy groups or linking the 3- and 4-position by dioxymethylene resulted in the inactive compounds **3a** and **3c**, respectively. While substitution at position 3- by a methoxy group and position 4- by a hydroxy group led to the moderately active compound **3b** with broad antitumor spectrum. Dealing with the 2-[(cycloalkylidene)-cyanomethyl]benzoxazole derivatives **4a** and **4b**, it was found that increasing the size of cycloalkane group from cyclopentyl to cyclohexyl resulted in obtaining a moderately active compound **4b** with broad spectrum of antitumor activity.

Our study was extended to a series of 2-(4-amino-3-substituted-2-thioxo-2,3-dihydrothiazol-5-yl)benzoxazoles **8a–d**. It was found that substitution at position-3 of the dihydrothiazole ring by butyl or 4-chlorophenyl group resulted in compounds **8a** and **8d**, respectively, possessing broad spectrum of antitumor activity. While substitution by benzyl or phenyl group led to the inactive compounds **8b** and **8c**, respectively.

With respect to the rigid tricyclic fused ring derivatives, substituted 3*H*-pyrido[2,1-*b*]benzoxazoles (**9–11**) were found to be devoid of antitumor activity.

3.2. In vitro anti-HIV-1 activity

Nine compounds 5; **6a**, **b**; **7a–c** and **11a–c** were selected by NCI and evaluated for their effects on HIV-1 induced cytopathogenicity in a human T_4 lymphocyte cell line (CEM) [38]. Activity is expressed as % of protection that represents the percentage of surviving HIV-infected cells treated with the test compound (at the indicated concentration) relative to the same uninfected untreated controls. The effective concentration 50% (EC_{50}) represents the concentration of the test agent resulting in 50% reduction of viral cytopathic effect. The 50% inhibitory concentration (IC_{50}), represent the toxic concentration of drug resulting in 50% growth inhibition of normal uninfected cells. The therapeutic index (TI_{50}) was determined by dividing (IC_{50}) by (EC_{50}). In this screen, the compounds are considered to be active if they display complete protection at a concentration < 0.1 μM. Compounds which show incomplete protection or show protection at a concentration above 0.1 μM are considered moderately active. As revealed from Table 4 compound **7a** showed moderate reduction of viral cytopathic effect by 36.6% at 2.0×10^{-5} M (IC_{50} 3.26×10^{-5}). While compound **7b** exhibited weak activity (24.6% at 2.0×10^{-5} M, IC_{50} 3.29×10^{-5}), its TI_{50} of **7b** is near unity. The other tested compounds were inactive.

3.3. Antimicrobial evaluation

The antimicrobial activity of the tested compounds in comparison with that of some control drugs is shown in Table 5. The results revealed that compounds **7b**, **c** showed activity against *Staphylococcus aureus* as Gram-positive bacteria lower than the compared control, ampicillin and streptomycin (minimal inhibitory concentration (MIC) values < 50 and < 25 μg ml^{-1}, respectively). Compounds **5** and **8a**, **d** were four times as active as streptomycin and about half that of ampicillin against the Gram-positive *Bacillus subtilis* (MIC < 12.5 μg ml^{-1}), while the activity of compounds **3b** and **7c** was two times as active as of streptomycin and one fifth that of ampicillin. Furthermore, determination of antibacterial activity against the Gram-negative enterobacter *P.*

Table 3
Selectivity ratios of the active compounds toward the nine tumor cell lines

Compound numbers	Subpanel tumor cell lines[a]								
	I	II	III	IV	V	VI	VII	VIII	IX
2a	0.72	0.89	0.59	1.61	0.54	1.32	0.82	1.23	0.79
2b	1.87	0.80	0.70	1.48	0.68	0.81	0.70	0.53	0.72
3b	0.74	1.09	1.03	1.02	1.08	1.10	0.76	0.81	1.03
4b	1.16	0.99	1.38	0.89	0.73	0.84	0.69	0.79	1.03
8a	0.51	0.97	0.91	1.04	0.89	1.10	0.94	1.12	1.00
8d	0.22	0.99	0.81	1.22	1.16	0.80	0.63	0.93	0.98

[a] I, Leukemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer.

Table 4
Maximum % protection, the corresponding doses (molar) and IC_{50} (molar) of the selected compounds

Compound numbers	NCS numbers	Maximum % protection	Dose (M)	IC_{50} (M)
5	722243-G/1	6.47	2.01×10^{-6}	4.02×10^{-6}
6a	722245-I/1	4.26	6.34×10^{-6}	$> 2.00 \times 10^{-4}$
6b	722231-R/1	10.24	2.00×10^{-5}	3.57×10^{-5}
7a	722244-H/1	36.61	2.00×10^{-5}	3.26×10^{-5}
7b	722230-Q/1	24.55	2.00×10^{-5}	3.29×10^{-5}
7c	722229-P/1	8.38	6.34×10^{-6}	3.69×10^{-5}
11a	722241-C/1	4.67	2.01×10^{-6}	$> 2.00 \times 10^{-4}$
11b	722242-F/1	4.27	6.35×10^{-7}	3.67×10^{-5}
11c	722228-O/1	5.78	2.00×10^{-5}	$> 2.00 \times 10^{-4}$

aurginosa indicated that compound **4b** was two times as active as ampicillin and streptomycin (MIC < 25 μg ml^{-1}), while compound **7c** exhibited activity equal to ampicillin and streptomycin (MIC < 50 μg ml^{-1}). Compounds **5**, **7b** and **11b** displayed half the activity of both control drugs (MIC < 100 μg ml^{-1}). Compounds **5** and **7c** exhibited significant activity against *Escherichia coli* but lower than that of ampicillin and streptomycin (half their activity, MIC < 25 μg ml^{-1}). The compounds were also tested against *Candida albicans* for their antimycotic activity. However, the results indicated that compound **7c** exhibited mild activity when compared with clotrimazole (MIC, < 100 μg ml^{-1}).

From the above study it has been revealed that the four benzoxazole derivatives: 2-[(4-hydroxy-3-methoxybenzylidene)cyanomethyl]benzoxazole **3b,** 2-(4-amino-3-butyl-2-thioxo-2,3-dihydrothiazol-5-yl)benzoxazole **8a** and 2-(4-amino-3-(4-chlorophenyl)-2-thioxo-2,3-dihydrothiazol-5-yl)benzoxazole **8d** exhibited modest activity only against Gram-positive bacteria *B. subtilis*. While compound 2-[(cyclohexylidene)cyanomethyl]benzoxazole **4b** was active against Gram-negative bacteria, *Pseudomonas aeruginosa* (two times as active as the reference drugs). On the other hand, compound 2-(4-oxothiazolidin-2-ylidene)methylbenzoxazole **5** and 2-[(N-4-chlorophenyl-2-thiocarbamoyl)cyanomethyl]-benzoxazole **7c** exhibited broader spectrum of activity against Gram-positive and Gram-negative bacteria.

By correlation of anticancer, anti-HIV and antibacterial results, compounds **3b**, **4b**, **5**, **7b**, **7c**, **8a** and **8d** have LC_{50}

Table 5
The inhibition zones (IZ) in mm diameter and MIC in μg ml^{-1} of benzoxazole derivatives

Compound numbers	*S. aureus*		*B. subtilis*		*P. aeruginosa*		*E. coli*		*C. albicans*	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
2a	–	–	–	–	15	–	12	–	–	–
2b	15	–	12	–	–	–	–	–	15	–
2c	15	–	–	–	15	–	12	–	–	–
2d	15	–	12	–	–	–	–	–	25	< 200
3a	–	–	10	–	–	–	–	–	15	–
3b	–	–	20	< 25	–	–	–	–	–	–
3c	–	–	10	–	–	–	–	–	15	–
4a	–	–	–	–	12	–	13	–	25	< 200
4b	14	–	13	–	22	< 25	20	< 100	24	< 200
5	20	< 100	28	< 12.5	20	< 100	22	< 25	–	–
6a	15	–	10	–	–	–	14	–	–	–
6b	17	–	12	–	–	–	18	–	–	–
7a	15	–	–	–	14	–	17	–	–	–
7b	23	< 50	19	–	20	< 100	17	–	–	–
7c	25	< 25	20	< 25	20	< 50	25	< 25	30	< 100
8a	–	–	25	< 12.5	–	–	–	–	–	–
8b	–	–	17	–	15	–	–	–	26	< 200
8c	–	–	17	–	14	–	10	–	15	–
8d	–	–	22	< 12.5	14	–	10	–	20	< 200
9a	–	–	15	–	15	–	10	–	–	–
9b	–	–	15	–	–	–	–	–	26	< 200
10	–	–	13	–	–	–	–	–	–	–
11a	–	–	–	–	–	–	12	–	–	–
11b	–	–	14	–	20	< 100	18	–	–	–
11c	–	–	–	–	15	–	15	–	–	–
Ampicillin	–	5	–	5	–	50	–	10	–	–
Streptomycin	–	10	–	50	–	50	–	10	–	–
Clotrimazole	–	–	–	–	–	–	–	–	–	5

(calculated from Tables 3 and 4; 27.27, 23.83, 0.93, 10.11, 12.10, 27.0 and 33.0 μg ml^{-1}, respectively). Accordingly, all the compounds with modest antibacterial activity are also cytotoxic at concentrations near their MIC's except compounds **8a** and **8d**.

It is worthy to mention that the riged tricyclic derivatives substituted 3*H*-pyrido[2,1-*b*]benzoxazoles (**9**–**11**) were devoid of anticancer, anti-HIV and anntimicrobial activities.

4. Experimental protocols

4.1. Chemistry

All melting points were determined in open-glass capillaries on a Gallenkamp melting point apparatus and are uncorrected. The IR spectra were recorded using KBr discs on a Perkin–Elmer 1430 spectrophotometer. ^{1}H-NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer or JNM-LA 400 FT NMR system using TMS as internal standard. MS were run on a Finnigan mass spectrometer model SSQ/7000 (70 eV). The microanalyses were performed at the Microanalytical Laboratory, National Research Center, Cairo, and the data were within ± 0.4% of the theoretical values.

*4.1.1. 2-[(Arylhydrazono)cyanomethyl]benzoxazoles (**2a–d**)*

To a solution of 2-cyanomethylbenzoxazole (**1**) (1.58 g, 10 mmol) in acetic acid (5 ml), an ice-cooled solution of the appropriate diazonium acetate solution [prepared by the addition of a solution of sodium nitrite (1 g, 15 mmol) in water (5 ml) to the required arylamine (10 mmol) in acetic acid (10 ml)] was added dropwise with stirring. Stirring was maintained for 30 min after which water was added and the precipitated product was filtered, washed with water, dried and crystallized from the proper solvent (Table 6).

IR of compounds **2a**–**d** (υ cm^{-1}): 3171–3066 (NH); 2226–2223 (C≡N); 1611–1599, 1551–1550, 1502–1481 (C=N, NH bending, C=C); 1278–1266, 1097–1087 (C–O–C).

^{1}H-NMR of compound **2a** (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 7.13–7.61 (m, 7H, 5 Ar–H and benzoxazole-$C_{5,6}$–H); 7.80 (distorted dd, 1H, benzoxazole-C_4–H); 7.93 (distorted dd, 1H, benzoxazole-C_7–H); 12.29 (s, 1/2H, NH, D_2O exchangeable); 13.42 (s, 1/2H, =N–NH, D_2O exchangeable). Electron Impact Mass Spectrum of compound **2a** *m/z* (% abundance): 263(6) M + 1; 262(34) M^+; 167(10); 158(7); 157(10); 150(6); 149(39); 129(15); 111(11); 109(10); 105(28); 98(9); 97(21); 96(9); 95(21); 93(12); 91(11); 87(7); 85(20); 84(14); 83(27); 82(13); 81(49); 79(8); 77(56); 72(29); 71(34); 70(19); 69(100); 68(16); 67(20); 60(28); 57(49); 56(16); 55(46).

^{1}H-NMR of compound **2b** (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 7.40–7.55 (m, 6H, 4 Ar–H and benzoxazole-$C_{5,6}$–H); 7.79 (dd, 1H, benzoxazole-C_4–H); 7.91 (distorted dd, 1H, benzoxazole-C_7–H); 12.34 (s, 1/2H, NH, D_2O exchangeable); 13.36 (s, 1/2H, =N–NH, D_2O exchangeable).

*4.1.2. 2-[(Arylidene)cyanomethyl]benzoxazoles (**3a–c**)*

To a stirred solution of 2-cyanomethylbenzoxazole (**1**) (0.63 g, 4 mmol) in absolute ethanol (10 ml), triethylamine (five drops) and the appropriate aldehyde (4 mmol) were added. The reaction mixture was stirred at room temperature for 3 h during which yellow crystals separated out. The crystalline product was filtered, washed with ethanol, dried and recrystallized from the proper solvent (Table 6).

IR of compounds **3a**–**c** (υ cm^{-1}): 2230–2223 (C≡N); 1588–1574, 1513–1502 (C=N, C=C); 1271–1240, 1180–1150, 1040–1022 (C–O–C). ^{1}H-NMR of compound **3a** ($CDCl_3$, δ ppm, Varian Gemini 200 MHz): 3.95, 3.97 (two s, each 3H, two OCH_3); 6.94 (d, 1H, Ar–C_5–H); 7.31–7.59 (m, 4H, Ar–C_6–H and benzoxazole-$C_{4,5,6}$–H), 7.76 (dd, J = 9.3 Hz, 1H, benzoxazole-C_7–H), 7.86 (s, 1H, Ar–C_2–H), 8.19 (s, 1H, =CH).

^{1}H-NMR of compound **3c** (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 6.11 (s, 2H, O–CH_2–O); 6.94 (d, J = 8.5 Hz, 1H, Ar–C_5–H); 7.36–7.59 (m, 4H, Ar–C_6–H and benzoxazole-$C_{4,5,6}$–H), 7.74–7.80 (m, 2H, benzoxazole-C_7–H and Ar–C_2–H), 8.19 (s, 1H, =CH).

Single-crystal X-ray analysis of compound **3b**: diffraction data of $C_{17}H_{12}N_2O_3$, mol. wt. 292.29, were collected from yellow crystals of dimensions 0.3 × 0.2 × 0.4 mm, using the automatic diffractometer Enraf Nonius FR590. The crystal system is orthorhombic with a = 13.7680 (9) Å, b = 9.9877 (6) Å, c = 21.1251 (13) Å, $\alpha = \beta = \gamma$ = 90.00 Å, V = 2904.9 (3) $Å^3$, Z = 8, D_{calc} = 1.337 g cm^{-3}. The space group is *Pbca*. Diffraction data: 1124 reflections were collected using Mo radiation λ (K_α) = 0.71073 Å. Computing data collection: Kappa CCD. The structure was solved by direct method using the program SHELXS-97. Agreement factor, R = 0.056.

Table 6
2-[Arylhydrazono)cyanomethyl]benzoxazoles (**2a**–**d**) and 2-[(arylidene)cyanomethyl]-benzoxazoles (**3a**–**c**)

Compound numbers	R	R_1	Yield (%)	M.p. (°C) (Cryst. Solv.)	Mol. formula (Mol. wt.)
2a	H	–	82	191–193 (EtOH)	$C_{15}H_{10}N_4O$ (262.27)
2b	Cl	–	86	195–197 (EtOH)	$C_{15}H_9ClN_4O$ (296.71)
2c	F	–	91	204–206 (Dioxane/EtOH)	$C_{15}H_9FN_4O$ (280.26)
2d	CH_3	–	81	204–206 (Dioxane/EtOH)	$C_{16}H_{12}N_4O$ (276.29)
3a	OCH_3	OCH_3	94	169–171 (EtOH)	$C_{18}H_{14}N_2O_3$ (306.32)
3b	OH	OCH_3	83	207–209 (EtOH)	$C_{17}H_{12}N_2O_3$ (292.29)
3c	OCH_2O		99	238–240 (Dioxane)	$C_{17}H_{10}N_2O_3$ (290.27)

*4.1.3. 2-[(Cycloalkylidene) cyanomethyl]benzoxazoles (**4a, b**)*

To a solution of 2-cyanomethylbenzoxazole (**1**) (1.58 g, 10 mmol) in absolute ethanol (10 ml), ammonium acetate (0.77 g, 10 mmol) and cyclopentanone or cyclohexanone (10 mmol) were added. The reaction mixture was heated under reflux for 2 h and left to cool to room temperature. The separated crystalline product was filtered, dried and recrystallized from ethanol.

Compound **4a**: m.p. 183–185 °C; yield 1.3 g (58%). IR (υ cm^{-1}): 2226 (C≡N); 1622, 1529, 1450 (C=N, C=C); 1243, 1066 (C–O–C). ^{1}H-NMR ($CDCl_3$, δ ppm, Varian Gemini 200 MHz): 1.67–1.99 (m, 4H, cyclopentyl-$C_{3,4}$–$\underline{H}_2$); 2.95, 3.17 (two t, each 2H, J = 6.4, 7.2 Hz, cyclopentyl-$C_{2,5}$–$\underline{H}_2$); 7.34–7.40 (m, 2H, benzoxazole-$C_{5,6}$–$\underline{H}$); 7.56 (dd, J = 9.2, 3 Hz, 1H, benzoxazole-C_4–$\underline{H}$); 7.75 (dd, J = 9.2, 3.1 Hz, 1H, benzoxazole-C_7–$\underline{H}$). $C_{14}H_{12}N_2O$ (224.26).

Compound **4b**: m.p. 88–90 °C; yield 1.55 g (65%). IR (υ cm^{-1}): 2227 (C≡N); 1598, 1547, 1448 (C=N, C=C); 1240, 1053 (C–O–C). ^{1}H-NMR ($CDCl_3$, δ ppm, Varian Gemini 200 MHz): 1.68–1.84 (m, 6H, cyclohexyl-$C_{3,4,5}$–H_2); 2.78, 3.13 (two t, each 2H, J = 6.6, 6.2 Hz, cyclohexyl-$C_{2,6}$–$\underline{H}_2$); 7.32–7.39 (m, 2H, benzoxazole-$C_{5,6}$–$\underline{H}$); 7.54 (distorted dd, 1H, benzoxazole-C_4–$\underline{H}$); 7.72 (distorted dd, 1H, benzoxazole-C_7–$\underline{H}$). $C_{15}H_{14}N_2O$ (238.29).

*4.1.4. 2-(4-Oxo-thiazolidin-2-ylidene)methylbenzoxazole (**5**)*

To a solution of 2-cyanomethylbenzoxazole (**1**) (1.58 g, 10 mmol) in absolute ethanol (5 ml), thioglycolic acid (0.92 g, 10 mmol) was added. The reaction mixture was heated under reflux for 7 h then left to cool to room temperature. The separated crystalline product was filtered, washed with ethanol, dried and recrystallized from ethanol (Table 7). IR (υ cm^{-1}): 3114 (NH); 1712 (C=O); 1631, 1551, 1450 (C=N, NH bending, C=C); 1243, 1209, 1136, 1002 (C–O–C, C–S–C). ^{1}H-NMR (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 3.95 (s, 2H, thiazolidinone-C_5–$\underline{H}_2$); 6.05 (s, 1H, =C$\underline{H}$); 7.27–7.32 (m, 2H, benzoxazole-$C_{5,6}$–$\underline{H}$); 7.58–7.66 (m, 2H, benzoxazole-$C_{4,7}$–$\underline{H}$); 11.68 (s, 1H, N$\underline{H}$, D_2O exchangeable).

*4.1.5. 2-[(3-Aryl-5-oxothiazolidin-2-ylidene) cyanomethyl]benzoxazoles (**6a, b**)*

To a well-stirred and ice-cooled suspension of finely powdered potassium hydroxide (0.56 g, 10 mmol) and 2-cyanomethylbenzoxazole (0.79 g, 5 mmol) in dry dimethylformamide (15 ml) the appropriate isothiocyanate (5 mmol) was added portionwise. After complete addition, stirring was continued at room temperature for 3 h. The reaction mixture was cooled to 0 °C, treated with chloroacetyl chloride (0.56 g, 5 mmol), stirred at room temperature for additional 6 h then poured onto ice/water. The resulting product was filtered, washed with water, dried and crystallized from the proper solvent (Table 7).

IR of Compound **6a** (υ cm^{-1}): 2205 (C≡N); 1743 (C=O); 1551, 1518, 1493 (C=N, C=C); 1222, 1200, 1066 (C–O–C, C–S–C).

IR of Compound **6b** (υ cm^{-1}): 2202 (C≡N); 1743 (C=O); 1547, 1526, 1490 (C=N, C=C); 1229, 1202, 1081 (C–O–C, C–S–C). ^{1}H-NMR (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 4.21 (s, 2H, thiazolidinone-C_4–$\underline{H}_2$); 7.38–7.42 (m, 2H, benzoxazole-$C_{5,6}$–$\underline{H}$); 7.63 (d, J = 8.8 Hz, 2H, Ar–$C_{2,6}$–$\underline{H}$); 7.67 (d, J = 8.8 Hz, 2H, Ar–$C_{3,5}$–$\underline{H}$); 7.73–7.77 (m, 2H, benzoxazole-$C_{4,7}$–$\underline{H}$).

*4.1.6. 2-[(N-substituted thiocarbamoyl) cyanomethyl]benzoxazoles (**7a–c**)*

To a well-stirred ice-cooled mixture of finely powdered potassium hydroxide (0.28 g, 5 mmol) and cyanomethylbenzoxazole (**1**) (0.79 g, 5 mmol) in dry dimethylformamide (15 ml) the appropriate isothiocyanate (5 mmol) was added portionwise. After complete addition, stirring was maintained at room temperature for 3 h Subsequently, the reaction mixture was poured onto ice/water and the mixture was acidified with 0.1 N HCl to pH 3-4. The resulting precipitate was filtered, washed with water, dried and crystallized from the proper solvent (Table 7).

IR of compounds **7a–c** (υ cm^{-1}): 3297–3233 (NH); 2203–2202 (C≡N); 1633–1629, 1533–1506, 1469–1466 (C=N, NH bending, C=C); 1602–1583, 1267–1253, 1191–1152, 966–933 (N–C=S amide I–IV bands).

^{1}H-NMR of compound **7a** (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 0.91 (t, J = 7 Hz, 3H, $(CH_2)_3$–C$\underline{H}_3$); 1.32

Table 7
2-(4-Oxo-thiazolidin-2-ylidene)methylbenzoxazole (5), 2-[(3-aryl-5-oxothiazolidin-2-ylidene)cyanomethyl]benzoxazoles (**6a**, **b**), 2-[(N-substituted thiocarbamoyl)cyanomethyl]benzoxazoles (**7a–c**) and 2-[(2-thioxo-2,3-dihydrothiazol-5-yl)benzoxazoles (**8a–d**)

Compound numbers	R	Yield (%)	M.p. (°C) (Cryst. Solv.)	Mol. formula (Mol. wt.)
5	–	52	202–204 (EtOH)	$C_{11}H_8N_2O_2S$ (232.26)
6a	C_6H_5	63	267–269 (Dioxane/EtOH)	$C_{18}H_{11}N_3O_2S$ (333.37).
6b	4-ClC_6H_4	92	255–257 (EtOH)	$C_{18}H_{10}ClN_3O_2S$ (367.81)
7a	$(CH_2)_3CH_3$	72	178–180 (EtOH)	$C_{14}H_{15}N_3OS$ (273.36)
7b	$CH_2C_6H_5$	95	228–230 (Dioxane)	$C_{17}H_{13}N_3OS$ (307.37)
7c	4-ClC_6H_4	99	211–213 (Dioxane)	$C_{16}H_{10}ClN_3OS$ (327.79)
8a	$(CH_2)_3CH_3$	52	191–193 (EtOH)	$C_{14}H_{15}N_3OS_2$ (305.42)
8b	$CH_2C_6H_5$	55	251–253 (Dioxane)	$C_{17}H_{13}N_3OS_2$ (339.44)
8c	C_6H_5	51	230–232 (EtOH)	$C_{16}H_{11}N_3OS_2$ (325.41)
8d	4-ClC_6H_4	56	242–244 (EtOH)	$C_{16}H_{10}ClN_3OS_2$ (359.86)

(m, 2H, CH_2–CH_2–CH_2–CH_3); 1.59 (m, 2H, CH_2–CH_2–CH_2–CH_2); 3.60 (t, J = 7.2Hz, 2H, CH_2–$(CH_2)_2$–CH_3); 7.28–7.66 (m, 5H, benzoxazole-$C_{4,5,6,7}$–H and CH); 8.84 (br.s, 1H, NH, D_2O exchangeable).

*4.1.7. 2-(4-Amino-3-substituted-2-thioxo-2,3-dihydrothiazol-5-yl)-benzoxazoles (**8a–d**)*

A mixture of 2-cyanomethylbenzoxazole (**1**) (1.58 g, 10 mmol), finely divided sulfur (0.32 g, 10 mmol) and triethylamine (1.4 ml, 10 mmol) in absolute ethanol (15 ml) was stirred at room temperature for 30 min. The appropriate isothiocyanate (10 mmol) was gradually added and stirring was continued for 1 h during which a yellowish green crystalline product separated out. The separated product was filtered, washed with ether, dried and crystallized from the proper solvent (Table 7).

IR of compounds **8a–d** (υ cm^{-1}): 3352–3157 (br.NH_2); 1628–1625, 1555–1552, 1451–1450 (C=N, NH bending, C=C); 1340–1326(C=S), 1248–1242, 1229–1223, 1023–1015 (C–O–C, C–S–C).

^{1}H-NMR of compound **8b** (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 5.62 (s, 2H, CH_2–C_6H_5); 7.23–7.41 (m, 7H, 5Ar–H and benzoxazole-$C_{5,6}$–H); 7.57–7.60 (m, 2H, benzoxazole-$C_{4,7}$–H); 7.63 (s, 2H, NH_2, D_2O exchangeable).

^{1}H-NMR of compound **8d** (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 7.17 (s, 2H, NH_2, D_2O exchangeable); 7.25–7.39 (m, 2H, benzoxazole-$C_{5,6}$–H); 7.55 (d, J = 8.8 Hz, 2H, Ar–$C_{2,6}$–H); 7.62–7.76 (m, 4H, Ar–$C_{3,5}$–H and benzoxazole-$C_{4,7}$–H).

*4.1.8. 3-Methyl (and phenyl)-1-oxo-1H-pyrido [2,1-b] benzoxazole-4-carbonitriles (**9a, b**)*

To a mixture of 2-cyanomethylbenzoxazole (**1**) (1.58 g, 10 mmol) and ammonium acetate (1.54 g, 20 mmol) in absolute ethanol (10 ml) ethyl acetoacetate (1.3 g, 10 mmol), or ethyl benzoylacetate (1.92 g, 10 mmol) was added. The reaction mixture was heated under reflux for 7 h and left to cool to room temperature. The separated crystalline product was filtered, washed with ether, dried and recrystallized from ethanol (Table 8).

IR of compound **9a** (υ cm^{-1}): 2215(C≡N); 1681 (C=O); 1526 (C=C); 1226, 1196, 1070 (C–O–C). ^{1}H-NMR ($CDCl_3$, δ ppm, Varian Gemini 200 MHz): 2.49 (s, 3H, CH_3); 6.28 (s, 1H, pyridobenzoxazole-C_2–H); 7.46–7.64 (m, 3H, pyridobenzoxazole-$C_{7,8,9}$–H), 8.53 (dd, J = 9.2, 2.4 Hz, 1H, pyridobenzoxazole-C_6–H).

IR of Compound **9b** (υ cm^{-1}): 2219(C≡N); 1683 (C=O); 1517 (C=C); 1274, 1196, 1039 (C–O–C).

*4.1.9. Ethyl (4-cyano-1-oxo-1H-pyrido[2,1-b]benzoxazole)-2-carboxylate (**10**)*

To a solution of 2-cyanomethylbenzoxazole (**1**) (1.58 g, 10 mmol) in absolute ethanol (5 ml), diethyl ethoxymethylenemalonate (2.16 g, 10 mmol) was added. The reaction mixture was heated under reflux for 7 h then left to cool to room temperature. The separated crystalline product was filtered, dried and recrystallized from dioxane (Table 8).

IR (υ cm^{-1}): 2228 (C≡N); 1727, 1695 (C=O ester, C=O amide); 1532 (C=C); 1242, 1173, 1017 (C–O–C). ^{1}H-NMR ($CDCl_3$, δ ppm, Varian Gemini 200 MHz): 1.41 (t, 3H, J = 7.2Hz, CH_2–CH_3); 4.42 (q, 2H, J = 7.2Hz, CH_2–CH_3); 7.54–7.73 (m, 3H, pyridobenzoxazole-$C_{7,8,9}$–H); 8.60 (s, 1H, pyridobenzoxazole-C_3–H), 8.66 (dd, J = 9.2, 3.2Hz, 1H, pyridobenzoxazole-C_6–H). The electron impact Mass Spectrum m/z (% abundance): 283(8) M + 1; 282(45) M^+; 238(16); 237(100); 211(14); 210(99); 209(10); 183(6); 182(49); 181(26); 154(6); 153(12); 127(13); 126(14); 103(6); 102(17); 101(7); 100(6); 90(7); 77(8); 76(15); 75(20); 64(29); 63(51); 62(12); 53(39); 52(11); 51(16).

*4.1.10. 1-Amino-3-aryl-3H-pyrido[2,1-b]benzoxazole-2,4-dicarbonitriles (**11a–c**)*

To a solution of 2-cyanomethylbenzoxazole (**1**) (1.58 g, 10 mmol) and piperidine (1 ml) in absolute ethanol (20 ml), the appropriate 2-arylidenemalononitrile (10 mmol) was added and the mixture was heated under reflux for 2–4 h. The separated crystalline product was filtered while hot, washed with ethanol then ethyl acetate, dried and recrystallized from the proper solvent (Table 8).

IR of compounds **11a–c** (υ cm^{-1}): 3462–3419, 3338–3305 (NH_2); 2198–2189 (C≡N); 1576–1571, 1483–1478 (NH bending, C=C); 1252–1242, 1089–1032 (C–O–C).

^{1}H-NMR of compound **11b** (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 4.73 (s, 1H, pyridobenzoxazole-C_3–H); 6.57 (s, 2H, NH_2, D_2O exchangeable); 7.23–7.46 (m, 7H, 4 Ar–H and pyridobenzoxazole-$C_{7,8,9}$–H); 7.73 (dd, J = 8.8, 2.5 Hz, 1H, pyridobenzoxazole-C_6–H).

^{1}H-NMR of compound **11c** (DMSO-d_6, δ ppm, Varian Gemini 200 MHz): 3.77 (s, 3H, OCH_3); 4.61 (s, 1H, pyridobenzoxazole-C_3–H); 6.48 (br.s, 2H, NH_2, D_2O exchangeable); 6.95 (d, J = 8.6 Hz, 2H, Ar–$C_{3,5}$–H); 7.24–7.26 (m, 2H, pyridobenzoxazole-$C_{7,8}$–H); 7.30 (d, J = 8.6 Hz, Ar–$C_{2,6}$–H); 7.49 (dd, J = 8, 2.4 Hz, 1H, pyridobenzoxazole-C_9–H); 7.72 (dd, J = 8.8, 2.2 Hz, 1H, pyridobenzoxazole-C_6–H).

Table 8
Substituted 1*H*-pyrido[2,1-*b*]benzoxazoles (**9a**, **b**; **10**; and **11a–c**)

Compound numbers	R	Yield (%)	M.p. (°C) (Cryst. Solv.)	Mol. formula (Mol. wt.)
9a	CH_3	56	220–222 (EtOH)	$C_{13}H_8N_2O_2$ (224.22)
9b	C_6H_5	53	235–237(EtOH)	$C_{18}H_{10}N_2O_2$ (286.28)
10	–	23	240–242 (Dioxane)	$C_{15}H_{10}N_2O_4$ (282.25)
11a	H	26	211–212 (Dioxane/H_2O)	$C_{19}H_{12}N_4O$ (312.33)
11b	Cl	29	207–209 (Dioxane)	$C_{19}H_{11}ClN_4O$ (346.77)
11c	OCH_3	28	217–219 (Dioxane)	$C_{20}H_{14}N_4O_2$ (342.35)

4.2. Biological evaluation

4.2.1. In vitro antineoplastic activity

The prepared compounds were tested for their in vitro anticancer activity against 60 human tumor cell lines, derived from nine clinically isolated types of cancer types (leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, breast cancer). Following the NCI preclinical antitumor drug discovery screen. Each compound was tested at five, 10-fold dilutions, 48 h continuous drug exposure protocol was used and a sulforodamine B (SRB) protein assay was used to estimate cell viability or growth [37].

4.2.2. In vitro anti-HIV-1 activity

The in vitro anti-HIV drug-testing system was performed in the national Cancer Institute's Developmental Therapeutics Program, AIDS antiviral screening program, according to a reported procedure [38]. The assay involved the killing of T_4 lymphocytes by HIV. T_4 lymphocytes (CEM cell line) were exposed to HIV at a virus-to-cell ratio of approximately 0.05 and treated with the compounds, dissolved in dimethylformamide, at doses ranging from 10^{-8} to 10^{-4}. A complete cycle of virus reproduction is necessary to obtain the required cell killing (incubation at 37 °C in a 5% carbon dioxide atmosphere for 6 days). Uninfected cells with the compound served as a toxicity control, whereas the infected and uninfected cells without the compound served as basic controls. After incubation, the tetrazolium salt XTT was added to all wells, and cultures were incubated to allow formazan color development by viable cells. Formazan production was measured spectrophotometrically and possible protective activity was confirmed by microscopic detection of viable cells. The effect of each compound on cell growth of HIV-infected and uninfected cells was compared to that of untreated uninfected cells. All tests were compared with AZT as positive control carried out at the same time under identical conditions.

4.2.3. IN vitro antimicrobial activity

The tested compounds were evaluated by the agar diffusion technique [39] using a 2 mg ml^{-1} solution in DMF. The test organisms were *S. aureus* (ATCC 6538) and *B. subtilis* (DB 100) as Gram-positive bacteria, *P. aeruginosa* (ATTC 27853) and *E. coli* (DH5a) as Gram-negative bacteria and *C. albicans* (0443P) as a representative for fungi. A control using DMF without the test compound was included for each organism. The MIC of the most active compounds was measured using the twofold serial broth dilution method [40]. Ampicillin, streptomycin and clotrimazol in DMF were used as reference drugs.

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