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Original article

Structure–activity relationships of novel heteroaryl-acrylonitriles as cytotoxic and antibacterial agents

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Abstract

Eighteen new 2,6-disubstituted acrylonitriles and two new (benzimidazol-1-yl)-acetamide derivatives were prepared and screened for antibacterial and cytotoxic activities on 12 human cancer cell lines. Based on the lead structure 2-(benzimidazol-2-yl)-3-(5-nitrothiophen-2-yl) acrylonitrile it was found that placement of methyl groups at the 5,6 positions of the benzimidazole ring lead to a 3-fold increase in overall cytotoxic activity. Replacing the nitrothiophene for pyridine reduced cytotoxic activity as did replacing the nitro group for a methoxy group. Cytotoxic activity was only slightly reduced when the benzimidazole ring was replaced by a imidazo[4,5-*b*]pyridine or a benzthiazole ring but replacement by benzoxazole led to a substantial decrease in activity. Moving the acrylonitrile group from position 2 to position 1 of the benzimidazole ring also resulted in moderately active compounds. (Benzimidazol-1-yl)acetamides showed only modest activity. The structure–activity relationships found in the cytotoxicity studies are mirrored in the results of the antibacterial experiments.

Keywords: Acrylonitriles; (Benzimidazol-1)acetamides; Cytotoxicity; Antibacterial; Structure–activity relationships; Anticancer

1. Introduction

Bactericidal and cytotoxic activities in human cancer cell lines have been ascribed to some substituted acrylonitriles. For example, various 2,3-disubstituted acrylonitriles were shown to have moderate tuberculostatic activity [1–5] while another group of acrylonitriles was described with antitrichomonal activity [6]. More recently, some acrylonitriles were found to be active against *Escherichia coli* and *Pseudomonas aeruginosa* [7]. Another example of acrylonitriles attached to an oxazolidinone moiety has been described with good antibacterial activity [8]. Acrylonitriles that act by either inhibition of tubulin polymerization [9] or EFG and PDGF tyrosine kinase activity [10,11] have been reported to be cytotoxic to cancer cells in vitro. In detailed structure–activity relationship studies, several members of a series of 3-aryl-2-benzotriazole acrylonitriles were recently reported to have potent in vitro antiproliferative activity [4,5].

Recently, we have reported that 3-arylacrylonitriles with either triazole [12,13] or benzimidazole [13] substituents in position 2 of the acrylonitirle also have good cytotoxic activity on human cancer cells. In particular, 2-benzimidazole substituted acrylonitriles were effective at inhibiting the growth of various human cancer cell lines in vitro. The most active compounds contained a 5-nitrofuran-2-yl or 5-nitrothiophen-2-yl ring at

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position 3 (e.g., compound **1**) and were 10-fold more potent than cisplatin at inhibiting cancer cell growth [13]. Herein we report on further structure–activity relationships in this class of compounds with respect to cytotoxic and antibacterial activities (for structures see Tables 1 and 2). In this work, compound **1** has been used as the lead structure and the nitrothiophene ring has been replaced with a biosteric pyridine ring. The importance of the benzimidazole group for biological activity has been explored as has the positioning of the acrylonitrile group on the imidazole ring. The effects of acrylonitriles on the progression of the cell cycle in cancer cells have also been investigated.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis

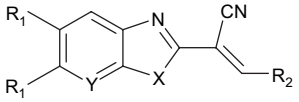
All novel 2-azolyl-3-heteroaryl-acrylonitriles **2**–**17** were prepared by the Knoevenagel condensation of (azol-2-yl)acetonitriles **I**–**V** with corresponding heteroaromatic aldehydes as depicted in Scheme 1.

Thus, 2-(benzimidazol-2-yl)acrylonitriles **2**–**8** were obtained upon treatment of the ethanolic solution of (benzimidazol-2-yl)acetonitrile (**I** or **II**) and aldehyde with catalytic amount of KOH at room temperature.

Analogous condensation of (3*H*-imidazo[4,5-*b*]pyridine-2-yl)acetonitrile **III** with aromatic aldehydes was previously found to proceed upon heating of the substrates in ethanol at reflux [14]. However, in our hands the reaction of **III** with heteroaromatic aldehydes carried out in boiling ethanol resulted in the formation of mixtures of tarry products, from which the desired acrylonitrile derivatives could not be isolated. It is well known that the Knoevenagel condensation is strongly solvent-dependent, and the dipolar aprotic solvents are especially useful for this reaction. Therefore, we used DMF as a solvent and piperidine as a catalyst. In this manner 2-(3*H*-imidazo[4,5-*b*]pyridine-2-yl)acrylonitriles **9**, **12** and **15** were formed at room temperature and could be separated from the reaction mixtures in 15–28% yields.

In turn, 2-(benzoxazol-2-yl)acrylonitriles **10**, **13** and **16** as well as 2-(benzothiazol-2-yl)acrylonitriles **11**, **14** and **17** were obtained in good yields by reacting acetonitriles **IV** and **V** with corresponding aldehydes in ethanol at ambient temperature in the presence of triethylamine (Scheme 1).

Table 1
Structures of compounds **1**–**17**



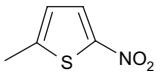
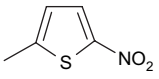
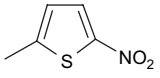
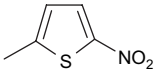
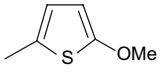
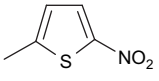
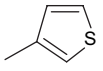
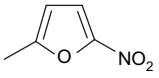
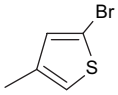
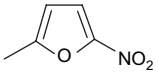
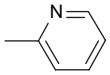
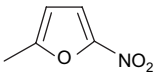
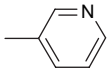
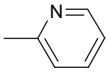
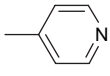
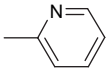
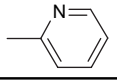
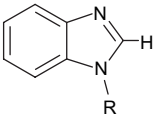
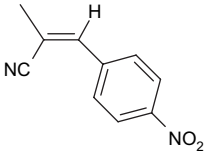
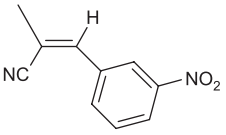
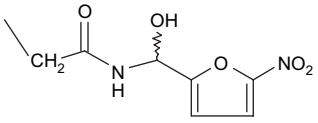
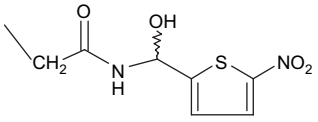
Compound	X	Y	R_1	R_2	Compound	X	Y	R_1	R_2
1	NH	CH	H		**9**	NH	N	H	
2	NH	CH	CH_3		**10**	O	CH	H	
3	NH	CH	H		**11**	S	CH	H	
4	NH	CH	H		**12**	NH	N	H	
5	NH	CH	H		**13**	O	CH	H	
6	NH	CH	H		**14**	S	CH	H	
7	NH	CH	H		**15**	NH	N	H	
8	NH	CH	H		**16**	O	CH	H	
					17	S	CH	H	

Table 2
Structures of compounds **18**–**21**



Compound	R
18	
19	
20	
21	

We then turned our attention to the positional analogues of the above acrylonitriles, i.e. 2-(benzimidazol-1-yl)acrylonitriles. As shown in Scheme 2, the reaction of (benzimidazol-1-yl)acetonitrile **VI** with aromatic aldehydes such as 4-nitro- and 3-nitrobenzaldehyde carried out in toluene at reflux in the presence of triethylamine gave the expected products **18** and **19** in 17 and 23% yield, respectively.

Encouraged by these results, we have tried the reaction of (benzimidazol-1-yl)acetamide (**VII**) with heteroaromatic aldehydes. We found, however, that the reaction of **VII** with either 5-nitrofuran carboxaldehyde or 5-nitrothiophene carboxaldehyde carried out in DMF solution at ambient temperature for 12 h in the presence of triethylamine did not furnish the expected Knoevenagel condensation products. Instead, careful examination of the IR and NMR spectra of the products **20** and **21** (Scheme 2) revealed that the addition of the amide NH group to aldehyde carbonyl group took place giving rise to the formation of products incorporating α-aminoalcohol moiety.

The IR spectrum of **21** contains a strong absorption characteristic of amide NH group at 3278 cm^{-1} and broad absorption in the range of 2625–3098 cm^{-1} attributable to associated OH as well as aromatic and aliphatic CH vibrations. Moreover, a strong band at 1670 cm^{-1} confirms the presence of amide CO group.

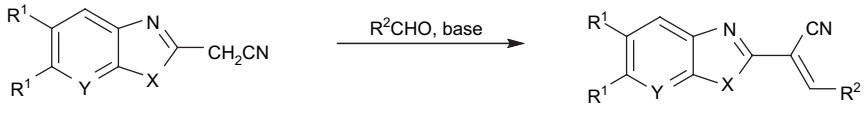
^{1}H NMR spectrum of **21** run at 500 MHz is also consistent with the proposed structure. Thus, the presence of two doublets centered at 5.04 ppm with $J = 16.5$ Hz may be assigned to N–CH_2 group in which the two protons are magnetically nonequivalent. Interestingly, the corresponding N–CH_2 group in unsubstituted substrate appears as a singlet at 4.9 ppm. At 6.47–6.50 ppm a multiplet of one proton appears due to the presence of CH group of hemiaminal moiety formed. Protons of thienyl ring appear as two doublets at 7.15 and 7.43 ppm; while protons of benzimidazole ring appear at 7.20–7.27 (m, 2H, C5-H, C6-H), 7.48 (d, 1H, C4-H, $J = 7.8$ Hz), 7.66 (d, 1H, C7-H, $J = 8.2$ Hz) and 8.2 ppm (s, 1H, C2-H). Moreover, two doublets at 8.07 ($J = 4$ Hz) and 9.54 ppm ($J = 8.8$ Hz) correspond to labile protons of OH and NH groups, respectively.

Also revealing is the ^{13}C NMR spectrum of **21**, that shows in the aliphatic region two signals at 47.35 and 70.91 ppm corresponding to the NCHOH and NCH_2 carbon atoms, respectively. In the aromatic region 11 signals in the range of 111.01–156.59 ppm are assigned to 7 benzimidazole and 4 thiophene carbon atoms. There is also one signal at 167.27 ppm attributable to the carbon atom of amide C=O group.

The aqueous solution stability of compound **21** was studied by reverse-phase HPLC in phosphate buffered saline at 37 °C. Beginning at a concentration of 20 μM, the relative amount of **21** decreased from 100% at time zero to 96, 94, 83 and 78% over the next 3, 24, 48 and 72 h, respectively. Thus, although **21** possess a hemiaminal structure, it is relatively stable in aqueous solution at pH 7.4.

2.1.2. Solubility studies

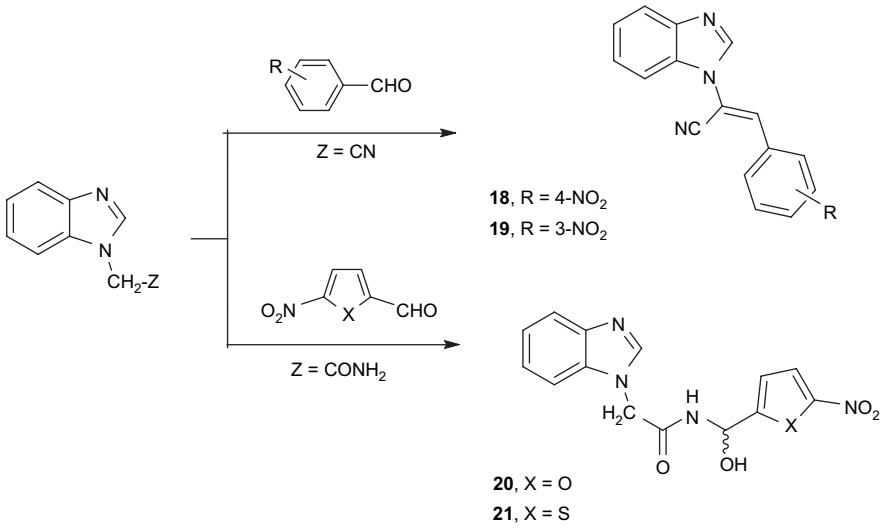
Table 3 shows the results of the solubility studies for selected compounds in phosphate buffered saline of pH 7.4 at room temperature. In general, the solubilities of the compounds in aqueous solutions were low. The compounds with the best water solubility were the imidazopyridine derivatives (**15** and **16**) that had 2-pyridino substituents at position 3 of the



I, X = NH, Y = CH, $R^1 = CH_3$
II, X = NH, Y = CH, R^1 = H
III, X = NH, Y = N, R^1 = H
IV, X = O, Y = CH, R^1 = H
V, X = S, Y = CH, R^1 = H

2-17

Scheme 1. Synthesis of 2-(azol-2-yl)acrylonitriles **2**–**17**.



Scheme 2. Synthesis of 1-substituted benzimidazoles **18**, **19**, and **20**, **21**.

acrylonitrile moiety. On the other hand, the oxazoles **10** and **13** and the benzothiazole **11** were practically insoluble under these conditions.

2.2. Pharmacology

2.2.1. Cytotoxicity studies

All compounds were investigated for cytotoxic activity in 12 human cancer cell lines while only selected ones were tested for antibacterial activity. The previously described compound **1** was used as the lead in these structure optimization studies [13]. Table 4 reports the IC_{50} values of the new compounds compared with known anticancer agents in up to 12 cell lines.

With respect to cytotoxic activity, placement of methyl groups at the 5,6 positions of the benzimidazole ring increased potency on the average by 3-fold. The IC_{50} values of **2** range between 0.02 and 0.2 μM (Table 4); thus, it is one of the most active antiproliferative acrylonitriles yet reported [4,5,13]. However, this compound has very poor water solubility, which has hindered its in vivo testing for antitumor activity. Therefore, we attempted the synthesis of analogues with other heterocyclics in the hope of improving water solubility. Replacing the benzimidazole ring with a imidazo [4,5-*b*]pyridine (compare **1** with **9**) or a benzthiazole ring (compare **1** with **11**) lead to only slight decreases in cytotoxic potency while replacement with an benzoxazole ring dramatically reduced potency (compare **1** with **10**, and **6** with **16**). This loss in activity could, in part, be due to the very low solubility of **10** and **13** in aqueous solutions (see above). However, compound **16** is one of the most water-soluble compounds in the series (Table 3).

Table 3
Water solubilities of saturated solutions of select compounds (in phosphate buffered saline, pH 7.4) at room temperature

Compound	λ_{max} (nm)	ε ($M^{-1}\,cm^{-1}$)	Solubility (μM)
9	420	24 556	0.5
10	387	14 476	<0.1
11	375	9500	<0.1
12	409	26 480	1.4
13	383	8454	<0.1
14	381	11 650	0.7
15	351	23 140	28
16	343	18 808	23
17	350	20 320	2.0

As reported in our previous work [13], acrylonitriles with a thiophene ring are more active than their furan ring counterparts (**9** with **12**, and **11** with **14**). The exception to this was with the benzoxazole pair **10** and **13**, where the furan analogue was actually more active than the thiophene counterpart.

The requirement for the 5-nitrothiophene ring on cytotoxic activity was explored to establish the importance of the nitro group for cytotoxic activity. Replacement of the nitro group with either a methoxy (**3**) or a bromo (**5**) group lead to a drastic reduction in activity, as previously described with other functional groups [13]. Bioisosteric replacement of the nitrothiophene with pyridine, which was substituted at either positions 2′, 3′ or 4′ of pyridine ring (compounds **6**–**8** and **15**–**17**), led to a reduction in potency; in general, replacement of the 5′-nitrothiophene ring with pyridine reduced activity by at least 10-fold (compare **1** with **7**, **9** with **15**, and **11** with **17**). While acrylonitriles with 2′- and 3′-substituted pyridines (i.e., **6** and **7**, respectively) showed modest cytotoxic activity, substitution of pyridine at the 4′ position reduced activity even more (i.e., **8**). Nevertheless, analogues with pyridine instead a nitrothiophene ring may be advantageous if systemic toxicity caused by the aromatic nitro group were to be eliminated. Furthermore, the compounds **15** and **16** showed an improvement in water solubility compared to their nitrothiophene and nitrofuran counterparts (see Table 3).

In previous work we showed that the replacement of the nitrothiophene ring by either a *p*-nitro or *m*-nitro substituted phenyl rings led to a dramatic loss in activity [13]. However,

Table 4
IC_{50} values (μM) in 12 human cancer cell lines[a]

Compound	Mean [b]	RSD[c]	RT-4	RT-112	5637	KYSE-70	KYSE-510	KYSE-520	YAPC	DAN-G	SISO	LCLC-103H	MCF-7	A-427
1	0.3	51.9	0.53	0.44	0.19	0.19	0.40	0.13	0.21	0.60	0.48	0.21	0.26	0.15
2	0.1	62.2	0.14	0.12	0.05	0.05	0.12	0.06	0.04	0.20	0.10	0.03	0.13	0.02
3	17.2	57.0	21.20	14.72	31.94	12.18	11.96	20.50	>40	13.81	36.84	5.24	13.25	7.19
4	15.2	48.5	7.82	13.33	14.07	16.04	23.04	14.55	28.95	15.66	23.77	3.75	14.44	6.39
5	>40	-	>40	>40	>40	>40	>40	>40	>40	>40	>40	22.76	>40	>40
6	5.1	53.7	2.68	4.73	2.28	6.82	3.67	10.54	6.58	3.67	1.91	7.42	8.14	2.84
7	3.5	28.9	3.47	3.50	1.79	4.25	3.92	5.13	4.92	3.27	3.02	1.97	3.15	3.29
8	30.9	23.9	40.14	30.70	31.22	37.15	>40	>40	>40	>40	>40	19.66	>40	26.25
9	0.4	57.8	0.33	0.62	0.17	0.38	0.74	0.32	0.30	0.62	0.53	0.11	0.20	0.11
10	7.8	80.8	6.57	3.88	3.91	5.30	22.63	2.76	1.70	12.62	14.12	12.06	5.69	2.33
11	0.7	29.4	0.92	0.75	0.62	0.51	0.93	0.46	0.42	0.72	0.64	0.88	0.83	0.38
12	1.5	85.2	1.76	1.11	1.08	1.33	5.46	1.58	1.02	1.81	0.72	0.41	1.39	0.75
13	2.7	59.1	3.98	1.41	1.65	2.91	7.00	1.68	2.86	3.00	2.86	1.33	2.87	1.13
14	2.7	45.7	4.30	1.78	2.37	2.38	5.44	2.00	2.25	3.84	2.50	1.47	3.33	1.21
15	7.9	32.0	10.04	8.18	3.90	8.14	13.88	7.98	8.89	7.97	5.53	7.37	8.28	5.15
16	>20	-	>20	nd	>20	nd	nd	nd	nd	nd	nd	>20	nd	nd
17	7.7	29.2	9.69	8.19	6.65	7.28	13.03	9.18	8.27	6.47	5.69	7.80	4.34	6.05
18	2.3	31.1	2.56	2.20	2.20	2.63	2.41	1.92	2.43	1.53	4.34	1.71	2.47	1.70
19	3.6	32.7	4.02	4.16	4.01	4.06	3.09	2.30	3.39	2.26	6.57	3.48	2.61	2.86
20	7.3	106.8	31.25	3.90	5.91	5.57	9.49	5.14	5.66	4.18	3.22	1.78	5.37	5.90
21	47.0	145.1	2.96	13.7	33.7	57.4	140	24.2	22.4	9.60	11.74	7.46	13.11	228
NF	11.4	88.6	7.0	nd	21.3	22.8	29.0	nd	nd	6.74	7.27	2.34	4.44	1.86
CDDP	1.6	86.3	1.61	1.22	0.35	0.63	0.44	3.61	4.09	0.73	0.24	0.90	1.38	1.96
Melph	6.1	83.9	14.25	4.69	0.31	16.16	8.18	10.49	5.95	2.65	1.00	4.00	3.71	5.13
Thtp	4.9	94.7	18.27	3.40	2.0	5.40	4.31	6.44	2.66	1.66	1.40	6.97	3.23	1.58

nd: not determined.
NF = nitrofurantoin, CDDP = cisplatin, Melph = melphalan, Thtp = thiotepa.
[a] Values are averages of 2–5 independent determinations. Individual values did not differ by more than 40%.
[b] Averaged IC_{50} values overall tested cancer cell lines.
[c] Relative standard deviation.

substitution of the nitrothiophene for a nitrobenzene ring and displacement of the acrylonitrile group to the nitrogen of the benzimidazole ring only reduced the cytotoxic potency by 10-fold (compare **1** with **18** and **19**). Thus, substitution of the acrylonitrile ring at either position 1 or 2 of the imidazole ring still leads to active compounds. Similarly, Carta and co-workers found that substitution at either nitrogen 1 or 2 of benzotriazole with a 3-arylacrylonitrile group gave substances that were cytotoxic [5].

Interestingly, the acrylonitrile group is not an absolute requirement for cytotoxic activity because benzimidazole *N*-methyl acetamides **20** and **21** are active too, albeit at much higher concentrations than the lead compound **1**.

The cytotoxic activities of new compounds were compared with the known antitumor agents, cisplatin, thiotepa and melphalan in Table 4. Because of the structural similarities of the new compounds with the antibacterial agent nitrofurantoin, this compound was also included in the cytotoxicity screen. Kamat and Lamm have recently shown that nitrofurantoin possess in vitro activity against three human transitional cell carcinoma lines [15]. This is confirmed by our findings that nitrofurantoin also possess modest activity in our panel of cell lines. However, the new acrylonitriles are substantially more active than nitrofurantoin, which is evidence that a nitrofuran ring alone is not responsible for cytotoxic activity.

A characteristic of anticancer drugs like cisplatin, melphalan and thiotepa in our panel of cell lines is that the relative standard deviation (RSD) of the cumulative IC_{50} values varies by at least 80% (Table 4). This is an indication that some cell lines are sensitive while other cells are intrinsically resistant to anticancer agents in vitro. However, toxic compounds show similar IC_{50} values overall cell lines (RSD < 80%). Thus, it was of interest to know if any of the acrylonitriles show RSD greater than 80%. As can be seen in Table 4, two of the acrylonitriles (i.e., **10** and **12**) have RSD greater than 80%. Interestingly, the two (benzimidazol-1-yl)acetamides **20** and **21** also show RSD greater than 80%, indicative of possible anticancer activity. While these four compounds were not the most potent ones, their specific activity against particular cell lines makes them of interest for further development as anticancer agents. Nevertheless, the majority of the compounds we tested showed no selectivity for any particular cell lines. It is noteworthy that most of the benzotriazole acrylonitriles described by Carta and co-workers also show a general lack of selectivity over five cancer cell lines [4,5].

2.2.2. Cell cycle studies

In previous work we found that compound **1** caused apoptosis in HL-60 human leukaemia cells by activating caspase 3 and 9 activities [13]. Thus, we were interested to know what effects, if any, this compound has on the cell cycle of HL-60 cells. Fig. 1 details the results of flow cytometry analysis that shows the distribution of cells in the G0/G1, S and G2/M phases after 24 and 72 h of treatment with various concentrations of **1**. After

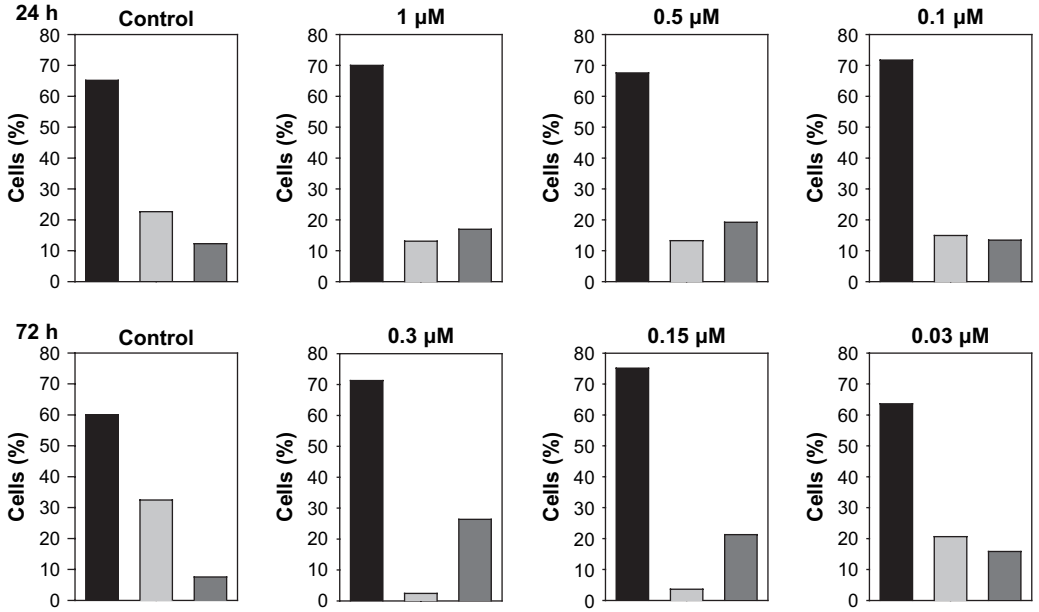


Fig. 1. Relative distribution of HL-60 cells after a 24 and 72 h treatment with compound **1** at various concentrations. Beginning on the left, the first column represents the fraction of cells in the G0/G1 phase, the second column is the fraction in the S phase and the third column is the fraction in the G2/M phase.

24 h very little effects on the distribution of cells are seen compared to untreated control, even at the highest concentration of 1 μM. However, by 72 h a substantial shift in the distribution is noticeable at concentrations at or above 0.15 μM, with a considerable increase in the distribution of cells in the G0/G1 and G2/M phases while the proportion in the S phase have decreased dramatically. These results are consistent with **1** causing a G1/S block in the cell cycle. Thus, **1** appears to act by a cell cycle specific mechanism, presumably in the G1 phase, to bring about apoptosis in HL-60 cells.

2.2.3. Antibacterial studies

The antibacterial activity of seven of the acrylonitriles was investigated on three different bacteria in culture: *Enterococcus hirae*, *Staphylococcus aureus* and *Staphylococcus epidermidis* (Table 5). The most sensitive and least sensitive of the acrylonitriles were the *S. epidermidis* and *E. hirae*, respectively. In fact, in *E. hirae*, all the tested compounds were practically inactive. Although not all the compounds were tested for antibacterial activity, those that were showed the same general structure–activity relationships were observed in the cytotoxicity experiments. For example, **1** and its imidazo[4,5-*b*] pyridine analogue **9** showed the best activity in the two *Staphylococcus* strains. Changing the imidazole ring of **1** to a thiazole (**11**) leads to a slight decrease in bactericidal activity. As observed with the cytotoxicity, replacement of the nitrothiophene ring for a pyridine ring (compare **1** with **6**) or replacement of the imidazole ring for an oxazole ring (compare **1** with **10**) leads to a major reduction in antibacterial activity. As with the cytotoxicity, the oxazole derivative with a nitrofuran ring was more active than with the imidazole (compare **10** and **13**) while the thiazole derivative with a nitrofuran ring was less active than the imidazole analogue (compare **11** and **14**). Thus, there may be some similarities in the mechanisms of action of both the cytotoxic and antibacterial activities. On the other hand, Carta and co-workers found no correlation between the antimycobacterial and cytotoxic activities in a series of 2-aryl-3-benzotriazole acrylonitriles [4].

Table 5
Antibacterial activity [MIC[a] and MBC[b]] of the selected acrylonitrile derivatives

Compound	*E. hirae*	*S. aureus*	*S. epidermidis*
1	>62.5 (>210)	1.9 (6.4)	0.95 (3.2)
6	>62.5 (>215)	>62.5 (>215)	62.5 (215)
9	62.5 (210)	1.9 (6.4)	0.95 (3.2)
10	>62.5 (>210)	62.5 (210)	62.5 (210)
11	>62.5 (>200)	3.9 (12)	3.9 (12)
13	>62.5 (>222)	31.25 (111)	31.25 (111)
14	>62.5 (>210)	>62.5 (>210)	>62.5 (>210)

[a] MIC = minimum inhibitory concentration in μg/ml (μM in parentheses).
[b] MBC = minimum bactericidal concentration μg/ml (μM in parentheses).

3. Conclusions

Acrylonitriles have specific effects on the cell cycle that lead to an accumulation of cells in the G2/M and G0/G1 phases. Earlier studies indicated that the cells undergo caspase activated apoptosis as a mechanism of cell death. The structure–activity relationships reported here indicate that heterocyclic replacements in both the benzene and imidazole rings of the benzimidazole group are tolerated but others are not. Thus, while the presence of the acrylonitrile unit is a requirement for good activity, its presence alone is not sufficient to kill cells. Changes in the nitrothiophene ring such as removal of the nitro group or replacement by a pyridine ring lead to strong decreases or even losses in activity. The position at

which the acrylonitrile group is attached to the imidazole ring is not essential for activity. Interestingly, very similar structure–activity relationships appear to be in effect for the antibacterial activity.

It is too early to speculate on the mechanism of action of these acrylonitriles. As we discussed previously [13], a number of mechanism could be responsible for the cytotoxic activity; e.g. 1,4-nucleophilic addition of thiols to the acrylonitrile double bond, redox-activity of the aromatic nitro group, or specific interactions with cellular receptors and enzymes. Additional work is needed to elucidate the mechanism of action.

Due to the poor water solubility of most of these compounds, attempts to test them for their in vivo antitumor activity have failed to date. Thus, the next goal of this project will be to develop water-soluble acrylonitriles, based on the SAR learnt in the current and previous studies [13].

4. Experimental

The following starting materials were obtained according to the previously described procedures: 2-(benzimidazol-2-yl)-3-(5-nitrothiophen-2-yl) acrylonitrile (**1**) [13], (5,6-dimethylbenzimidazol-2-yl)acetonitrile (**I**) [16], (benzimidazol-2-yl)acetonitrile (**II**) [17], (3*H*-imidazo[4,5-*b*]pyridine-2-yl)acetonitrile (**III**) [18], (benzoxazol-2-yl)acetonitrile (**IV**) [19], (benzothiazol-2-yl)acetonitrile (**V**) [20], (benzimidazol-1-yl)acetonitrile (**VI**) [8] and (benzimidazol-1-yl)acetamide (**VII**) [21].

Melting points were determined on a Boetius 545 apparatus and are not corrected. IR spectra were recorded on a Perkin–Elmer 1600 FTIR spectrometer using a mixture of the compound and KBr. UV and UV–vis spectra were taken on the Specord M40 (Jena Carl Zeiss, Germany). ^{1}H and ^{13}C NMR spectra were measured with Varian Gemini Plus spectrometer in DMSO-d_6 at 500 and 125 MHz, respectively. The chemical shift values (δ) are expressed in parts per million relative to tetramethylsilane as internal standard and coupling constants (J) are in hertz. All reagents were used directly as obtained commercially.

4.1. General method for preparation of 2-(benzimidazol-2-yl)acrylonitriles (2–8)

To a warm solution of corresponding (benzimidazol-2-yl) acetonitrile (0.01 mol) in absolute ethanol (20 ml) was added suitable carboxaldehyde (0.01 mol) and five drops of 10% methanolic KOH solution. The reaction mixture was stirred at room temperature for 0.5 h, and then the crude product that precipitated was collected by suction and purified by recrystallization from suitable solvent.

According to the above procedure the following acrylonitriles were prepared.

4.1.1. 2-(5,6-Dimethylbenzimidazol-2-yl)-3-(5-nitrothiophen-2-yl)acrylonitrile (2)

Yield 51%, m.p. 335–340 °C. UV–vis (methanol): λ_{max} = 209, *439* nm (ε = 18 197). IR (KBr) ν = 3318 (NH), 2230 (CN), 1578, 1530 (NO_2), 1499, 1442, 1386 (NO_2), 1227, 1037 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ = 2.33 (s, 6H, 2 × CH_3), 7.32 (s, 1H, benzimidazole), 7.46 (s, 1H, benzimidazole), 7.8 (d, J = 4.4 Hz, 1H, C3′-H thiophene), 8.23 (d, J = 4.4 Hz, 1H, C4′-H thiophene), 8.49 (s, 1H, =CH), 13.0 (s, 1H, NH) ppm. HPLC: t_R = 15.53 min (λ = 439 nm). Anal. ($C_{16}H_{12}N_4O_2S$ (324.34)) C: calcd 59.24, found 59.12; H: calcd 3.73, found 3.96; N: calcd 17.27, found 17.26; S: calcd 9.8, found 9.08.

4.1.2. 2-(Benzimidazol-2-yl)-3-(5-methoxythiopen-2-yl)acrylonitrile (3)

Yield 28%, m.p. 217–220 °C. UV–vis (methanol): λ_{max} = *208*, 398 nm (ε = 29 512). IR (KBr) ν = 3299 (NH), 2217 (CN), 1623, 1591, 1534, 1481, 1327, 1050 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ = 4.0 (s, 2H, OCH_3), 6.6 (d, 1H, thiophene, J = 4.3 Hz), 715–7.45 (m, 2H, benzimidazole), 7.6 (d, 1H, thiophene, J = 4.3 Hz), 7.6–7.8 (m, 2H, benzimidazole), 12.8 (br, 1H, NH) ppm. HPLC: t_R = 6.93, 10.48 min (1:2 λ = 346 nm). Anal. ($C_{15}H_{11}N_3SO$ (281.32)) C: calcd 64.04, found 62.1; H: calcd 3.94, found 4.2; N: calcd 14.93, found 14.6; S: calcd 11.37, found 11.18.

4.1.3. 2-(Benzoimidazol-2-yl)-3-(3-thiophen-3-yl)-acrylonitrile (4)

Yield 51%, m.p. 241 °C. UV–vis (methanol): λ_{max} = 208, *348* nm (ε = 20 892). IR (KBr) ν = 3092 (NH), 2972, 2114, 2223 (CN), 1504, 1441, 1330, 1050 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ = 7.18–7.32 (m, 2H, benzimidazole), 7.53 (d, J = 7.7 Hz, 1H, C4′-H thiophene), 7.65 (d, J = 7.7 Hz, 1H, C5′-H thiophene), 7.88–7.80 (m, 2H, benzimidazole), 8.36 (m, 3H, =CH, C2′-H thiophene), 13.04 (s, 1H, NH) ppm. HPLC: t_R = 6.35 min (λ = 346 nm). Anal. ($C_{14}H_9N_3S$ (251.3)) C: calcd 66.91, found 63.77; H: calcd 3.61, found 4.16; N: calcd 16.72, found 16.04; S: calcd 12.73, found 12.22.

4.1.4. 2-(Benzimidazol-2-yl)-3-(5-bromothiophen-3-yl)-acrylonitrile (5)

Yield 1.52 g, 51%, m.p. 256–260 °C. UV–vis (methanol): λ_{max} = 208, *355* nm (ε = 27 542). IR (KBr) ν = 3267 (NH) 3094, 4046, 2236 (CN), 1597, 1434, 1407, 1332, 1276, 1208 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ = 7.29–7.21 (m, 2H, benzimidazole), 7.63–7.59 (m, 2H, benzimidazole), 7.88 (d, J = 1.7 Hz, 1H, C2′-H thiophene), 8.26 (s, 1H, =CH), 8.34 (d, J = 1.7 Hz, 1H, C4′-H thiophene), 13.07 (s, 1H, NH) ppm. HPLC: t_R = 10.48 min (λ = 346 nm). Anal. ($C_{14}H_8N_3SBr$ (330, 208)) C: calcd 50.92, found 51.09; H: calcd 2.4, found 2.46; N: calcd 12.72, found 13.14; S: calcd 9.60, found 9.81.

4.1.5. 2-(Benzimidazol-2-yl)-3-(pyridin-2-yl)acrylonitrile·C_2H_5OH (6)

Yield 18%, m.p. 203–207 °C. UV–vis (methanol): λ_{max} = 208, *355* nm (ε = 24 547). IR (KBr) ν = 3063, 2969, 2868, 2228 (CN), 1577, 1560, 1470, 1442, 1423, 1317, 1232, 1317, 1050 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ = 1.05 (triplet, 3H, CH_3 ethanol), 3.44 (m, 2H, CH_2 ethanol), 4.36 (triplet, 1H, OH ethanol), 7.27 (s, 2H, benzimidazole), 7.55 (m, 1H, C5′-H pyridine), 7.70 (s, 1H, benzimidazole), 7.58 (s, 1H,

benzimidazole), 7.86 (d, 1H, C3′-H pyridine), 8.02 (m, 1H, C4′-H pyridine), 8.38 (s, 1H, =C–H) 8.80 (d, 1H, C6′-H pyridine), 13.16 (s, 1H, NH) ppm. ^{13}C NMR 147.97 ppm (C-2′), 127.17 (C-3′), 138.24 (C-4′), 126.31 (C-5′), 150.43 (C-6′), 144.23 (=CH), 105.57 (=*C*–CN), 116.22 (C≡N), 151.43 (C-2) ppm. HPLC: $t_R = 4.77$ min ($\lambda = 346$ nm). Anal. ($C_{17}H_{16}N_4O$ (292.10)) C: calcd 69.8, found 69.78; H: calcd 5.47, found 5.45; N: calcd 19.17, found 19.10.

4.1.6. 2-(Benzimidazol-2-yl)-3-(pyridin-3-yl)-acrylonitrile (7)

Yield 7%, m.p. 251–253 °C. UV–vis (methanol): $\lambda_{max} =$ *208*, 348 nm ($\varepsilon = 19\,054$). IR (KBr) $\nu = 3073$, 2987, 2929, 2225 (CN), 1588, 1436, 1414, 1313 cm^{-1}. ^{1}H NMR (DMSO-d_6) $\delta = 7.27$–7.25 (m, 2H, benzimidazole), 7.64–7.61 (m, 3H, benzimidazole, C5′-H pyridine), 8.37 (s, 1H, =CH), 8.44 (dd, $J_{ortho} = 7$ Hz, $J_{meta} = 5$ Hz, 1H, C4 pyridine), 8.70 (d, $J = 5$ Hz, 1H, C6 pyridine), 9.00 (d, 1H, C2′-H pyridine), 13.17 (s, 1H, NH) ppm. HPLC: $t_R = 4.64$ min ($\lambda = 346$ nm). Anal. ($C_{15}H_{10}N_4$ (246.12)) C: calcd 73.02, found 73.3; H: calcd 4.09, found 4.17; N: calcd 22.74, found 22.60.

4.1.7. 2-(Benzimidazol-2-yl)-3-(pyridin-4-yl)acrylonitrile (8)

Yield 10%, m.p. 249–260 °C. UV–vis (methanol): $\lambda_{max} =$ *206*, 351 nm ($\varepsilon = 19\,000$). IR (KBr) $\nu = 3273$ (NH), 3027, 2241 (CN), 1587, 1544, 1436, 1414, 1313, 1249 cm^{-1}. ^{1}H NMR (DMSO-d_6) $\delta = 7.29$–7.26 (m, 2H, benzimidazole), 7.66–7.64 (m, 2H, benzimidazole), 7.82 (d, $J = 6$ Hz, 2H, pyridine), 7.34 (s, 1H, =CH), 8.80 (d, $J = 6$ Hz, pyridine), 13 (br s, 1H, NH) ppm. HPLC: $t_R = 4.67$ min ($\lambda = 346$ nm). Anal. ($C_{15}H_{10}N_4$ (246.12)) C: calcd 73.02, found 69.94; H: calcd 4.09, found 4.14; N: calcd 22.74, found 22.39.

4.2. General procedure for the preparation of 2-(3H-imidazo[4,5-b]pyridin-2-yl)acrylonitriles (9, 12 and 15)

A solution of 2-(3*H*-imidazo[4,5-*b*]pyridine-2-yl)acetonitrile (0.2 g, 1.26 mmol) and the corresponding carboxaldehyde (1.26 mmol) in anhydrous DMF (3 ml) was treated with three drops of piperidine and the reaction mixture was stirred at room temperature for 0.5 h. Then, the reaction mixture was cooled to 5 °C (ice bath) and kept at this temperature for 10 min. The product that precipitated was collected by filtration and washed with methanol.

According to the above procedure the following acrylonitriles were obtained.

4.2.1. 2-(3H-Imidazo[4,5-b]pyridin-2-yl)-3-(5-nitrothiophen-2-yl)acrylonitrile (9)

Yield 15%, m.p. 270–273 °C (dec.). UV–vis, λ_{max} (DMF) = 421 nm, λ_{max} (methanol) = 409 nm, λ_{max} (Dulbecco's buffer) = 409 nm ($\varepsilon = 24\,556$). IR (KBr) $\nu = 3055$ (NH), 2372, 2218 (CN), 1654 (C=C), 1582, 1494 (NO), 1432, 1335 (NO), 1261, 1224, 1080, 817 cm^{-1}. ^{1}H NMR (DMSO-d_6) $\delta = 13.78$ (s, 1H, NH), 8.64 (s, 1H, CHO, DMF), 8.42 (dd, 1H, 6H pyridine, $J_{ortho} = 4.8$ Hz, $J_{meta} = 1.4$ Hz), 8.26 (d, 4′H thiophene, $J = 4.4$ Hz), 8.12 (br d, 1H, 4H pyridine, $J_{ortho} = 7.8$ Hz), 7.95 (s, 1H, H-β), 7.85 (d, 1H, 3′H thiophene, $J = 4.4$ Hz), 7.3 (dd, 1H, 5H pyridine, $J_{5,4} = 7.8$ Hz, $J_{5,6} = 4.8$ Hz), 2.88 (s, 3H, CH_3 DMF), 2.73 (s, 3H, CH_3 DMF). Anal. ($C_{13}H_7N_5O_2S$ (297.27) × DMF (73.09)) C: calcd 52.26, found 51.99; H: calcd 3.8, found 3.22; N: calcd 22.86, found 24.86; S: calcd 8.7, found 9.57.

4.2.2. 2-(3H-Imidazo[4,5-b]pyridin-2-yl)-3-(5-nitrofuran-2-yl)acrylonitrile (12)

Yield 28%, m.p. 274–278 °C (dec). UV–vis, λ_{max} (DMF) = 409 nm, λ_{max} (methanol) = 396 nm, λ_{max} (Dulbecco's buffer) = 409 nm ($\varepsilon = 26\,480$). IR (KBr) $\nu = 3128$ (NH), 3091, 2231 (CN), 1677 (C=C), 1591, 1558, 1524 (NO), 1466, 1411, 1350 (NO), 1258, 1030, 964, 933, 811 cm^{-1}. ^{1}H NMR (DMSO-d_6) $\delta = 13.7$ (s, 1H, NH), 8.44 (d, 1H, Ar-H, $J = 4$ Hz), 8.31 (s, 1H, CHO), 8.13 (d, 1H, Ar-H, $J = 8$ Hz), 7.95 (s, 1H, HC=), 7.90 (d, 1H, furan, $J = 4$ Hz), 7.59 (d, 1H, furan $J = 4$ Hz), 7.29–7.36 (m, 1H, Ar-H), 2.88 (s, 3H, CH_3 DMF), 2.73 (s, 3H, CH_3 DMF). Anal. ($C_{13}H_7N_5O_3 \cdot 2H_2O$ (317.23)) C: calcd 49.17, found 47.41; H calcd 3.46, found 3.67; N: calcd 22.06, found 22.74.

4.2.3. 2-(3H-Imidazo[4,5-b]pyridin-2-yl)-3-(pyridin-2-yl)acrylonitrile (15)

Yield 27%, m.p. 156–158 °C. UV–vis, λ_{max} (DMF) = 355 nm, λ_{max} (methanol) = 349 nm, λ_{max} (Dulbecco's buffer) = 351 nm ($\varepsilon = 23\,140$). IR (KBr) $\nu = 3079$ (NH), 3004, 2918, 2849, 2232 (NH), 1576, 1527, 1437, 1284, 1117, 944 cm^{-1}. ^{1}H NMR (DMSO-d_6) $\delta = 13.6$ (s, 1H, NH), 8.83 (d, 1H, pyridine, $J = 4$ Hz), 8.44 (s, 1H, HC=). 8.42 (d, 1H, Ar-H, $J = 1$ Hz), 7.95–8.12 (m, 1H, pyridine), 7.87 (d, 1H, Ar-H, $J = 8$ Hz), 7.53–7.59 (m, 1H, pyridine), 7.32 (d, 1H, pyridine, $J = 1$ Hz), 7.28–7.35 (m, 1H, Ar-H). Anal. ($C_{14}H_9N_5$ (247.25)) C: calcd 68.01, found 67.49; H: calcd 3.67, found 4.31; N: calcd 28.32, found 26.12.

4.3. General procedure for preparation of 2-(benzoxazol-2-yl)acrylonitriles (10, 13 and 16) and 2-(benzothiazol-2-yl)acrylonitriles (11, 14 and 17)

A solution of (benzoxazol-2-yl)acetonitrile or (benzothiazol-2-yl)acetonitrile (0.35 g, 2 mmol) and corresponding carboxaldehyde (2 mmol) in ethanol (5 ml) was treated with five drops of triethylamine. The reaction mixture was stirred at room temperature for 0.5 h, and then solid that precipitated was separated by suction, washed with ethanol and purified by crystallization from DMF.

According to the above procedure the following compounds were prepared.

4.3.1. 2-(Benzoxazol-2-yl)-3-(5-nitrothiophen-2-yl)-acrylonitrile (10)

Yield 78%, m.p. 307–308 °C (dec.). UV–vis, λ_{max} (DMF) = 413 nm, λ_{max} (Dulbecco's buffer) = 387 nm ($\varepsilon = 14\,476$). IR (KBr) $\nu = 3109$, 3030, 2922, 2232 (CN), 1607, 1583, 1419, 1495 (NO), 1450, 1337 (NO), 1227,

1051, 815 cm^{-1}. ^1H NMR (DMSO-d_6) $\delta = 8.3$ (s, 1H, =CH), 8.08 (m, 1H, Ar-H), 7.88 (m, 1H, Ar-H), 7.82 (d, 1H, thiophene, $J = 8$ Hz), 7.83 (d, 1H, thiophene, $J = 8$ Hz), 7.47–753 (m, 2H, Ar-H). Anal. ($C_{14}H_7N_3O_3S$ (297.09)) C: calcd 56.56, found 56.22; H: calcd 2.37, found 2.53; N: calcd 14.13, found 14.14; S: calcd 10.79, found 10.79.

4.3.2. 2-(Benzoxazol-2-yl)-3-(5-nitrofuran-2-yl) acrylonitrile (**13**)

Yield 85%, m.p. 264–268 °C. UV–vis, λ_{max} (DMF) = 398 nm, λ_{max} (methanol) = 389 nm, λ_{max} (Dulbecco's buffer) = 383 nm ($\varepsilon = 8454$). IR (KBr) $\nu = 3154$, 3056, 2237 (CN), 1621, 1561, 1520, 1460 (NO), 1408, 1349 (NO), 1265, 1045, 1018, 967, 818 cm^{-1}. ^1H NMR (DMSO-d_6) $\delta = 8.46$ (s, 1H, HC=), 7.73–7.9 (m, 2H, Ar-H), 7.9 (d, 1H, furan, $J = 4$ Hz), 7.65 (d, 1H, furan, $J = 4$ Hz), 7.4–7.6 (m, 2H, Ar-H). Anal. ($C_{14}H_7N_3O_4$ (281.22)) C: calcd 59.79, found 59.17; H: calcd 2.51, found 2.73; N: calcd 14.94, found 14.66.

4.3.3. 2-(Benzoxazol-2-yl)-3-(pyridin-2-yl)acrylonitrile (**16**)

Yield 51%, m.p. 155–158 °C. UV–vis, λ_{max} (DMF) = 342 nm, λ_{max} (methanol) = 340 nm, λ_{max} (Dulbecco's buffer) = 343 nm ($\varepsilon = 18\,088$). IR (KBr) $\nu = 3061$, 2231 (CN), 1577, 1560, 1471, 1428, 1341, 1247, 1033, 992 cm^{-1}. ^1H NMR (DMSO-d_6) $\delta = 8.83$ (d, 1H, C3-H pyridine, $J = 4$ Hz), 8.55 (s, 1H, HC=), 8.01–8.05 (m, 1H, C5′-H pyridine), 7.99 (d, 1H, C6′-H pyridine, $J = 8$ Hz), 7.88 (d, 1H, Ar-H, $J = 8$ Hz), 7.83 (d, 1H, Ar-H, $J = 4$ Hz), 7.57–7.60 (m, 1H, C4′-H pyridine), 7.47–7.53 (m, 2H, Ar-H). Anal. ($C_{15}H_9N_3O$ (247.25)) C: calcd 72.87, found 72.46; H: calcd 3.67, found 3.94; N: calcd 16.99, found16.89.

4.3.4. 2-(Benzothiazol-2-yl)-3-(5-nitrothiophen-2-yl)-acrylonitrile (**11**)

Yield 91%, m.p. 260–262 °C (dec). UV–vis, λ_{max} (DMF) = 405 nm, λ_{max} (methanol) = 411 nm, λ_{max} (Dulbecco's buffer) = 375 nm ($\varepsilon = 9500$). IR (KBr) $\nu = 3103$, 3029, 2222 (CN), 1527, 1503, 1456 (NO), 1337 (NO), 1221, 1039, 939, 815 cm^{-1}. ^1H NMR (DMSO-d_6) $\delta = 8.75$ (s, 1H, HC=), 8.03–8.23 (m, 4H, Ar-H), 7.57–7.61 (m, 2H, thiophene). Anal. ($C_{14}H_7N_3O_2S_2$ (313.35)) C: calcd 53.66, found 53.67; H: calcd 2.25, found 2.50; N: calcd 13.41, found 13.39; S: calcd 20.47, found 20.38.

4.3.5. 2-(Benzothiazol-2-yl)-3-(5-nitrofuran-2-yl)-acrylonitrile (**14**)

Yield 43%, m.p. 248–250 °C (dec). UV–vis, λ_{max} (DMF) = 405 nm, λ_{max} (methanol) 395 nm, λ_{max} (Dulbecco's buffer) = 381 nm ($\varepsilon = 11\,650$). IR (KBr) $\nu = 3191$, 3043, 2226 (CN), 1554, 1515, 1463 (NO), 1393, 1347 (NO), 1260, 1016, 969, 935 cm^{-1}. ^1H NMR (DMSO-d_6) $\delta = 8.40$ (s, 1H, HC=), 8.25 (d, 1H, Ar-H, $J = 7$ Hz), 8.13 (d, 1H, Ar-H, $J = 8$ Hz), 7.89 (d, 1H, furan, $J = 4$ Hz), 7.59–7.65 (m, 2H, Ar-H), 7.55 (d, 1H, furan, $J = 4$ Hz). Anal. ($C_{14}H_7N_3O_3S$ (297.29)) C: calcd 56.56, found 56.33; H: calcd 2.37, found 2.48; N: calcd 14.13, found 14.19; S: calcd 10.79, found 10.55.

4.3.6. 2-(Benzothiazol-2-yl)-3-(pyridin-2-yl)-acrylonitrile (**17**)

Yield 42%, m.p. 152–155 °C. UV–vis, λ_{max} (DMF) = 342 nm, λ_{max} (methanol) 340 nm, λ_{max} (Dulbecco's buffer) = 350 nm ($\varepsilon = 20\,320$). IR (KBr) $\nu = 3052$, 2992, 2217 (CN), 1557, 1469, 1433, 1313, 1171, 980 cm^{-1}. ^1H NMR (DMSO-d_6) $\delta = 8.82$ (d, 1H, C3′-H pyridine, $J = 4$ Hz), 8.47 (s, 1H, HC=), 8.19–8.23 (m, 2H, Ar-H), 8.09–8.13 (m, 1H, C5′-H pyridine), 8.06 (d, 1H, C6′-H pyridine, $J = 4$ Hz), 7.95–8.02 (m, 2H, Ar-H), 7.50–7.65 (m, 1H, C4′-H pyridine). Anal. ($C_{15}H_9N_3S$ (263.32)) C: calcd 68.42, found 68.43; H: calcd 3.45, found 3.79; N: calcd 15.96, found 15.94; S: calcd 12.18, found 11.73.

4.4. Preparation of 2-(benzimidazol-1-yl)-3-(4-nitrophenyl)-acrylonitrile (**18**)

A solution of (benzimidazol-1-yl)acetonitrile (0.3 g, 1.9 mmol), 4-nitrobenzaldehyde (0.3 g, 1.98 mmol) and triethylamine (0.5 ml) in toluene (3 ml) was heated at reflux for 5 h. The volatile material was evaporated under reduced pressure and the solid residue was washed thoroughly with methanol and acetone to give 0.13 g (yield 23%) of the final product **7**; m.p. 177–179 °C. IR (cm^{-1}): 3089, 2223, 1593, 1522, 1452, 1345, 1291, 1233. ^1H NMR (DMSO-d_6) $\delta = 7.38$–7.52 (m, 2H, CH), 7.84–7.89 (m, 2H, CH), 8.18 (d, 2H, CH, $J = 8.5$ Hz), 8.27 (s, 1H, CH), 8.48 (d, 2H, CH, $J = 8.5$ Hz), 8.67 (s, 1H, CH).

4.4.1. 2-(Benzimidazol-1-yl)-3-(3-nitrophenyl)-acrylonitrile (**19**)

Compound **19** was prepared according to the above procedure using 3-nitrobenzaldehyde. Yield 0.1 g (17%), m.p. 180–182 °C, IR (cm^{-1}): 3107, 3059, 2215, 1623, 1531, 1350, 1281, 1229. ^1H NMR (DMSO-d_6) $\delta = 7.37$–7.52 (m, 2H, CH), 7.83–7.90 (m, 3H, CH), 7.97 (d, 1H, CH, $J = 8.06$), 8.28 (s, 1H, CH) 8.36–8.47 (m, 2H, CH), 8.65 (s, 1H, CH), 8.81–8.82 (m, 1H, CH).

4.5. Preparation of 2-(1H-benzimidazol-1-yl)-N-[(hydroxy-(5-nitrofuran-2-yl)methyl)]-acetamide (**20**)

A solution of (benzimidazol-1-yl)acetamide (0.8 g, 0.05 mole) and equimolar amount of 5-nitrofuran-2-carboxaldehyde and triethylamine in DMF (5 ml) was stirred at room temperature for 12 h. Then the solvent was evaporated to dryness under reduced pressure, and the semisolid residue thus obtained was treated with a mixture of methanol, acetone and water in a ratio of 5:1:1 (5 ml) to give pure product **9** in the form of a grey precipitate which was collected by suction. Yield 0.3 g (22%), m.p. 129–133 °C. IR (cm^{-1}): 3287, 3136, 3094, 2853, 2730, 1655, 1533, 1503, 1354, 1244, 1096, 1072, 1020, 811, 744.

4.5.1. 2-(1H-Benzimidazol-1-yl)-N-[(hydroxy-(5-nitrothiophen-2-yl)methyl)]-acetamide (**21**)

Compound **21** was prepared according to the above procedure using 5-nitrothiophene-2-carboxaldehyde. Yield 0.44 g (30%), m.p. 137–140 °C. IR (cm^{-1}): 3278, 3098, 3053, 2852, 2625, 1670, 1541, 1499, 1329, 1163, 1085, 1030, 741. ^{1}H NMR (DMSO-d_6) $\delta = 5.04$ (dd, 2H, CH_2, $J = 16.5$ Hz), 6.47–6.50 (m, 1H, CH hemiaminal), 7.15 (d, 1H, CH thienyl), 7.43 (d, 1H, CH thienyl), 7.20–7.27 (m, 2H, C5, 6-H benzimidazole), 7.48 (d, 1H, C4-H benzimidazole, $J = 7.8$ Hz), 7.66 (d, 1H, C7-H benzimidazole, $J = 8.2$ Hz) and 8.2 ppm (s, 1H, C2-H benzimidazole), 8.07 (d, 1H, OH, $J = 4$ Hz), 9.54 (d, 1H, NH, $J = 8.8$ Hz). ^{13}C NMR (DMSO-d_6) $\delta = 47.3$, 70.9, 11.0, 120.0, 122.2, 123.0, 125.2, 130.7, 135.0, 143.8, 145.6, 150.4, 156.5, 167.2. RP-HPLC: chromatography with a Nucleosil 120-5C18 column (250 × 4 cm) with a mobile phase of 20% methanol in 20 mM phosphate buffer (pH 3.3) at a flow rate of 0.7 ml/min gave a single peak with a retention time of 9.92 min (detection at $\lambda = 270$ nm).

4.6. Solubility studies

Substances were added in solid form to Dulbecco's phosphate buffered saline (pH 7.4) in excess of their expected solubility and stirred in the dark for 24 h at room temperature. An aliquot of the suspension was then filtered through a 0.22 mm filter and the absorption of the filtrate was measured at the λ_{max} (see Table 3) for each of the compounds with a Spekol 1200 (Analytik Jena, FRG). The extinction coefficients of the compounds at the λ_{max} were used to calculate the concentrations of the dissolved substances in the filtrate.

4.7. Cytotoxic activity

All cell lines were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) (Braunschweig, Germany). Cytotoxicity studies were performed with a well-established microtiter assay based on the staining of adherent cells with crystal violet; the method has been described in detail in previous publications [13,22,23]. DMF stock solutions of the compounds were diluted 1000-fold in cell culture medium (RPMI 1640 medium supplemented with 10% FCS) to give the final test concentration. Cells were continuously exposed to compounds for 96 h at 37 °C in a humid atmosphere of 5% CO_2/air. The IC_{50} values were estimated by least squares analysis of the dose–response curves to give the concentration of substance that inhibits cell growth by 50% compared to untreated controls. Reported IC_{50} values are the averages of 2–5 independent determinations.

4.8. Cell cycle analysis

HL-60 cells were treated with the concentrations of compound **1** shown in Fig. 1 without previously synchronizing the cells. After 24 and 72 h the fractions of cells in the various phases of the cycle were measured. Cells were fixed in 70% ice-cold ethanol. Cellular DNA content, indicating if cells are in cell cycle phase G0/G1, S or G2/M, was quantified by using propidium iodide and flow cytometry analysis (FACS Calilbur; Becton Dickinson, Karlsruhe, Germany) following standard protocols.

4.9. Antibacterial activity

Antibacterial activity was investigated in vitro on bacterial strains *E. hirae* (NCTC10541), *S. aureus* (NCTC4163) and *S. epidermidis* (NCTC14990). Overnight bacterial culture was diluted with Mueller–Hinton broth to the density of 105 CFU/ml. Tested compounds were dissolved in DMSO and diluted (in geometric progression) to the concentration 0.24–62.5 μg/ml with Mueller–Hinton broth. Then, each tube was inoculated with the bacterial suspension and incubated at 37 °C for 24 h. The lowest concentration at which there was no visible growth was taken as the minimal inhibitory concentration (MIC). In addition, 100 ml of suspension from each tube that showed no growth were inoculated in Mueller–Hinton agar plates to control for bacterial viability. The minimal bactericidal concentration (MBC) was defined as the minimal concentration of compounds required to kill of the organisms in the medium after 24 h incubation [24].

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