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Short communication

Synthesis and antiproliferative activity of 3-aryl-2-(1*H*-benzotriazol-1-yl)acrylonitriles. Part III

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Abstract

A new series of 30 3-aryl-2-(1*H*-benzotriazol-1-yl)acrylonitriles were synthesized and tested for biological activity as part of our research in the antimicrobial and antitumor fields. In particular, title compounds were evaluated in vitro against representative strains of Gram-positive and Gram-negative bacteria (*S. aureus*, *Salmonella spp*), mycobacteria (*M. fortuitum*, *M. smegmatis* ATCC 19420 and *M. tuberculosis* ATCC 27294), yeast and mould (*C. albicans* ATCC 10231 and *A. fumigatus*). Furthermore, their antiretroviral activity against HIV-1 was determined in MT-4 cells together with cytotoxicity. In these assays title compounds and 47 additional derivatives described previously (P. Sanna, A. Carta, M.E. Rahbar Nikookar, Eur. J. Med. Chem. 35 (2000) 535–543; P. Sanna, A. Carta, L. Gherardini, M.E. Rahbar Nikookar, Farmaco 57 (2002) 79–87) were tested for their capability to prevent MT-4 cell growth. All compounds resulted devoid of antibacterial, antifungal and anti-HIV-1 activity. In anti-mycobacterial assays several compounds resulted active ($MIC_{50} = 6.0–70$ μM) against *M. tuberculosis*. However, since they showed cytotoxicity against MT-4 cells at lower concentrations ($CC_{50} = 0.05–25$ μM), their anti-mycobacterial activity was not selective. For this reason, the most cytotoxic compounds were also evaluated for antiproliferative activity against a panel of human cell lines derived from both hematological and solid tumors. Compound **34** resulted the most potent compound against the above human tumor-derived cell lines.

Keywords: 3-Aryl-2-(1*H*-benzotriazol-1-yl)acrylonitriles; Antiproliferative acivity; Skin melanoma; Breast adenocarcinoma

1. Introduction

Recently, we reported the synthesis and the anti-mycobacterial activity of over 50 3-substituted-2-[1*H*(2*H*)-benzotriazol-1(2)-yl]acrylonitriles, prop-2-enamides and propenoic acids [1,2]. Several compounds showed an interesting activity in a preliminary screening against *M. tuberculosis* within an international program with the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF). As part of our antimicrobial and antitumor research programs [3–6] we synthesized a new series of 30 3-aryl-2-(1*H*-benzotriazol-1-yl)acrylonitriles and evaluated their biological activity in order to extend previous SAR studies. Substituents at position 4 in the phenyl moiety (I, OCH_3 and CN) were chosen with the aim to complete the previous series. Furthermore, the substituents typical of previous derivatives (F, Cl, Br, CF_3 and NO_2), along with new substituents such as I, OCH_3 and CN, were introduced either at positions 2 or 3, whereas F, Cl and CF_3 were contemporaneously introduced at two different positions. Both the previously synthesized [1,2] and the new derivatives were evaluated for cytotoxicity against MT-4 cells, carried out in parallel with anti-HIV-1 activity, in order to determine whether the compounds were endowed with selective antimicrobial/antiviral activity.

Due to the relevant cytotoxicity shown by many derivatives, we studied in detail their potential antipro-

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liferative activity against a panel of cell lines derived from hematological and solid tumors.

2. Chemistry

The synthesis of compounds **4**–**17**, **48**–**73**, and **75**–**78** has been previously reported [1,2] and their chemical structures are shown in Fig. 1 and Table 1. The synthesis of the new series of 3-aryl-2-(1*H*-benzotriazol-1-yl)acrylonitriles **18**–**47**, depicted in Fig. 1 and Table 1, was accomplished as previously reported [1,2] by straightforward condensation of the key intermediate 2-(benzotriazol-1-yl)acetonitrile (**1**) [1] with the appropriate commercially available aldehydes, or prepared as reported in the literature. In this new series, among the two possible geometric isomers (*E*/*Z*), only *E*-isomers were obtained as the sole product.

Spectral (IR, UV–vis, ^{1}H-NMR) and analytical (elemental analyses, MS) data of all the new compounds are in accordance with those of the previously described counterparts [1,2] and support the assigned chemical structure.

3. Microbiology

The new compounds were evaluated in vitro against representative strains of Gram-positive and Gram-negative bacteria (*S. aureus*, *Salmonella spp*), various mycobacterial strains (*M. fortuitum*, *M. smegmatis* ATCC 19420 and *M. tuberculosis* ATCC 27294), and yeast and mould strain (*C. albicans* ATCC 10231 and *A. fumigatus*). Streptomycin, Ciprofloxacin, Ofloxacin, Isoniazid, Rifampicin and Miconazole were used as reference drugs. Title compounds were also evaluated for anti-HIV-1 activity in MT-4 cells. All derivatives, no matter whether belonging to new or previously synthesized series, were tested for cytotoxicity in MT-4 cells and the most active compounds were then evaluated against a panel of human cell lines derived from hematological (CCRF-CEM, WIL-2NS and CCRF-SB) and solid (SKMEL28, MCF7, SKMES-1, HepG2, and DU145) tumors. In this case, two antitumor agents with different mode of action, 6-mercapto-purine (6MP) and Etoposide, were used as reference drugs.

4. Results and discussion

The new compounds **18**–**47** reported in Fig. 1 and Table 1 were evaluated against representative strains of Gram-positive, Gram-negative bacteria, mycobacteria, yeasts and moulds. Because of an ongoing screening program carried out to identify new antiretroviral compounds, the new derivatives were also evaluated for anti-HIV-1 activity in MT-4 cells. However, none of these compounds showed antibacterial and antifungal activity or the capability to protect the HIV-1-infected cells from the virus-induced cytopathic effect (data not shown). When tested against atypical mycobacterial strains these compounds resulted inactive with the sole exception of **46** and **47**, which showed slight activity against *M. smegmatis* ($MIC_{50} = 86.3–93.6$ μM). Although several derivatives showed significant activity against *M. tuberculosis* ($MIC_{50} = 6.0–70$ μM), they could not be considered as selective inhibitors because they resulted in cytotoxicity in MT-4 cells at concentrations very close to those active against Mycobacteria (Table 2).

Compounds related to title derivatives, i.e. the 3-substituted-2-(1*H*(2*H*)-benzotriazol-1(2)-yl)acryloni-

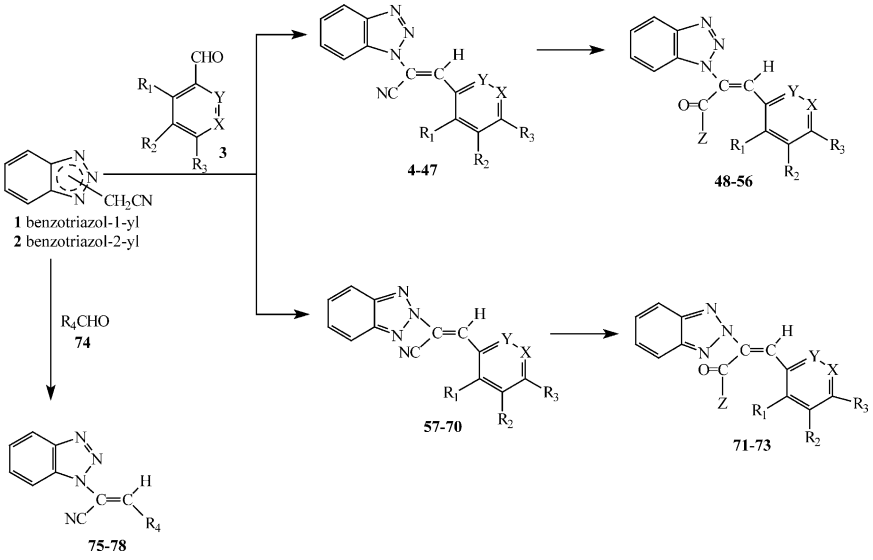

Fig. 1. Structures of the compounds (**4**–**73** and **75**–**78**).

Table 1
Substituents of the compounds (**4**–**73** and **75**–**78**) and related aldehydes

Compound	R_1	R_2	R_3	X	Y	Z	R_4	Reference
4	H	H	H	CH	CH	–	–	[1]
5	H	H	CH_3	CH	CH	–	–	[1]
6	H	H	F	CH	CH	–	–	[1]
7	H	H	Cl	CH	CH	–	–	[1]
8	H	H	Br	CH	CH	–	–	[1]
9	H	H	CF_3	CH	CH	–	–	[1]
10	H	H	CO_2H	CH	CH	–	–	[1]
11 [a]	H	H	NO_2	CH	CH	–	–	[1]
12	H	OCH_3	OCH_3	C–OCH_3	CH	–	–	[2]
13	H	OCH_3	OCH_3	CH	CH	–	–	[2]
14	H	O–CH_2–O		CH	CH	–	–	[2]
15	H	H	H	none	NH	–	–	[2]
16	H	H	H	none	O	–	–	[2]
17 [a]	H	H	H	none	S	–	–	[2]
18	F	H	H	CH	CH	–	–	–
19	Cl	H	H	CH	CH	–	–	–
20	Br	H	H	CH	CH	–	–	–
21	I	H	H	CH	CH	–	–	–
22	CF_3	H	H	CH	CH	–	–	–
23	NO_2	H	H	CH	CH	–	–	–
24	OCH_3	H	H	CH	CH	–	–	–
25	H	F	H	CH	CH	–	–	–
26	H	Cl	H	CH	CH	–	–	–
27	H	Br	H	CH	CH	–	–	–
28	H	I	H	CH	CH	–	–	–
29	H	CF_3	H	CH	CH	–	–	–
30	H	NO_2	H	CH	CH	–	–	–
31	H	OCH_3	H	CH	CH	–	–	–
32	H	CN	H	CH	CH	–	–	–
33	H	H	I	CH	CH	–	–	–
34	H	H	OCH_3	CH	CH	–	–	–
35	H	H	CN	CH	CH	–	–	–
36	Cl	Cl	H	CH	CH	–	–	–
37	Cl	H	Cl	CH	CH	–	–	–
38	Cl	H	H	CH	C–Cl	–	–	–
39	H	Cl	Cl	CH	CH	–	–	–
40	H	Cl	H	C–Cl	CH	–	–	–
41	F	F	H	CH	CH	–	–	–
42	F	H	F	CH	CH	–	–	–
43	F	H	H	CH	CH	–	–	–
44	H	F	F	CH	CH	–	–	–
45	H	F	H	C–F	CH	–	–	–
46	CF_3	H	CF_3	CH	CH	–	–	–
47	H	CF_3	H	C–CF_3	CH	–	–	–
48	H	H	H	CH	CH	OH	–	[2]
49	H	H	Cl	CH	CH	OH	–	[2]
50	H	H	Br	CH	CH	OH	–	[2]
51	H	H	CF_3	CH	CH	OH	–	[2]
52	H	H	H	CH	CH	NH_2	–	[2]
53	H	H	CH_3	CH	CH	NH_2	–	[2]
54	H	H	Cl	CH	CH	NH_2	–	[2]
55	H	H	Br	CH	CH	NH_2	–	[2]
56	H	H	CF_3	CH	CH	NH_2	–	[2]
57	H	H	H	CH	CH	–	–	[1]
58	H	H	CH_3	CH	CH	–	–	[1]
59	H	H	F	CH	CH	–	–	[1]
60	H	H	Cl	CH	CH	–	–	[1]
61	H	H	Br	CH	CH	–	–	[1]
62	H	H	CF_3	CH	CH	–	–	[1]
63	H	H	CO_2H	CH	CH	–	–	[1]
64	H	H	NO_2	CH	CH	–	–	[1]
65	H	OCH_3	OCH_3	C–OCH_3	CH	–	–	[2]

Table 1 (*Continued*)

Compound	R_1	R_2	R_3	X	Y	Z	R_4	Reference
66	H	OCH_3	OCH_3	CH	CH	–	–	[2]
67	H	$O–CH_2–O$		CH	CH	–	–	[2]
68 [a]	H	H	H	none	NH	–	–	[2]
69	H	H	H	none	O	–	–	[2]
70	H	H	H	none	S	–	–	[2]
71	H	H	H	CH	CH	NH_2	–	[2]
72	H	H	CH_3	CH	CH	NH_2	–	[2]
73	H	H	Cl	CH	CH	NH_2	–	[2]
75	H	H	H	CH	CH	–	cyclohexyl	[2]
76	H	H	H	CH	CH	–	4-diphenyl	[2]
77	H	H	H	CH	CH	–	α-naphtyl	[2]
78	H	H	H	CH	CH	–	β-naphtyl	[2]

[a] Also *Z* isomer.

triles (**4**–**17**, **48**–**73**, and **75**–**78**) shown in Fig. 1 and Table 1, have been recently reported to posses antimycobacterial activity in collaboration with TAACF [1,2]. However, since the evaluation of cytotoxicity was not carried out, we tested previous and present acrylonitriles for cytotoxicity against MT-4 cells. As shown in Tables 2 and 3, some of new and their previously synthesized compounds (**5**, **7**, **8**, ***Z*** **11**, **14**, **30**, **34**, **50**, **64**, and **78**) inhibited the MT-4 growth at sub-micromolar concentration ($CC_{50} = 0.05–0.5$ μM). In addition to above compounds, an isomer of **34** (**31**) and some dichloro derivatives (**36**, **38**, and **40**) were then evaluated against a panel of human cell lines derived from hematological and solid tumors (Tables 4 and 5). It is noteworthy that the compound **34**, bearing a methoxy group at position 4 of the phenyl moiety, potently inhibits the proliferation of cells derived from hematological and solid human tumors, resulting 5–100-fold more potent than 6-MP. Moreover, it showed 6–10-fold greater potency than Etoposide against skin melanoma and breast adenocarcinoma. On the other hand, its counterpart with a methoxy group at position 3 (**31**) is both less potent and selective.

5. Conclusion

Acrylonitrile derivatives are endowed with potent antiproliferative activity, whereas, as far as the antimycobacterial activity is concerned, it does not appear to be selective. Since compound **34** resulted active against both hematological and solid human tumors, it might represent a new lead compound which could be further optimized.

6. Experimental

Melting points were determined by a Kofler hot stage or Digital Electrothermal apparatus, and are uncorrected. Infrared spectra are for Nujol mulls and were recorded using a Perkin–Elmer 781 spectrophotometer. UV spectra are qualitative and were recorded in nm for solutions in EtOH with a Perkin–Elmer Lambda 5 spectrophotometer. The abbreviations used are as follows: sh for shoulder, infl for inflection. ^{1}H-NMR spectra were recorded on a Varian XL-200 (200 MHz) instrument, using TMS as internal standard. The chemical shift values are reported in ppm (δ) and coupling constants (J) in Hertz (Hz). Signal multiplicities are represented by: s (singlet), d (doublet), dd (double doublet), m (multiplet), and br s (broad singlet). MS spectra were performed on combined HP 5790–HP 5970 GC/MS apparatus. Column chromatography was performed using 230–400 mesh silica gel (Merck silica gel 60). Light petroleum refers to the fraction with bp 40–60 °C. Elemental analyses were performed by the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, University of Padua (Italy). The analytical results for C, H, N and chlorine, when present, were within ±0.4% of the theoretical values.

6.1. Intermediates

2-(Benzotriazol-1-yl)acetonitrile (**1**) was prepared following the procedure previously described [1]. 4-Iodobenzaldehyde was prepared as reported in the literature [7]. We also found it convenient to use the latter method for the synthesis of the 2- and 3-iodobenzaldehyde; the spectroscopic and analytical data of the products obtained were in accordance with those reported in literature [8,9].

6.2. General procedure for preparation of *E*-2-(*1H*-benzotriazol-1-yl)-3-arylacrylonitriles (*18*–*47*)

To a solution of 2-(*1H*-benzotriazol-1-yl)-acetonitrile **1** (6.3–15.8 mmol) and Et_3N (16.0–37.0 mmol) in $C_6H_5CH_3$ (25–30 mL) stirred at room temperature (r.t.) for 15–20 min, was added dropwise a solution of

Table 2
Anti-mycobacterial activity and cytotoxicity of compounds (**18**–**47**)

Compound	CC_{50} [a]	MIC_{50} [b]\MIC_{90} [c]		
	MT-4	*M. tuberculosis*	*M. smegmatis*	*M. fortuitum*
18	2.5	47.2/89.4	> 100	> 100
19	2.0	13.8/28.9	> 100	> 100
20	2.0	9.0/33.1	> 100	> 100
21	25.0	11.3/28.2	> 100	> 100
22	5.0	22.4/65.8	> 100	> 100
23	31.0	> 100	> 100	> 100
24	8.3	32.3/80.2	> 100	> 100
25	3.6	55.3/ > 100	> 100	> 100
26	3.0	51.2/ > 100	> 100	> 100
27	3.0	29.6/ > 100	> 100	> 100
28	3.0	58.2/90.9	> 100	> 100
29	18.0	11.4/69.5	> 100	> 100
30	0.4	38.2/ > 100	> 100	> 100
31	4.7	52.7/ > 100	> 100	> 100
32	1.5	20.0/64.5	> 100	> 100
33	1.4	17.0/33.3	> 100	> 100
34	0.05	70.0/ > 100	> 100	> 100
35	3.0	> 100	> 100	> 100
36	4.0	> 100	> 100	> 100
37	6.0	> 100	> 100	> 100
38	5.0	6.0/22.2	> 100	> 100
39	5.8	≥ 100	> 100	> 100
40	2.0	63.8/ > 100	> 100	> 100
41	3.3	52.4/92.4	> 100	> 100
42	38	> 100	> 100	> 100
43	2.8	24.7/ > 100	> 100	> 100
44	2.5	54.6/ > 100	> 100	> 100
45	2.0	56.9/98.6	> 100	> 100
46	11.0	9.3/24.4	86.3/ > 100	> 100
47	8.4	6.9/26.3	93.6/ > 100	> 100
Ciprofloxacin	60.0	1.4/3.3	0.6/2.6	2.7/11.9
Ofloxacin	> 100	1.3/3.4	0.9/2.6	0.7/5.1
Isoniazid	> 100	0.1/5.1	1.2/6.5	> 100
Rifampicin	> 100	0.2/0.9	1.6/7.5	n.d.

[a] Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method.
[b] Minimum inhibitory concentration (μM) required to reduce the number of viable Mycobacteria by 50%, as determined by the MTT method.
[c] Minimum inhibitory concentration (μM) required to reduce the number of viable Mycobacteria by 90%, as determined by the MTT method. Data represent mean values for two separate experiments. Variation among duplicate samples was less than 15%.

Table 3
Cytotoxicity of compounds (**4**–**17**, **48**–**73** and **75**–**78**)

Compounds	CC_{50} [a] MT-4	Compounds	CC_{50} [a] MT-4
4	4.0	**56**	4.0
5	0.5	**57**	12.0
6	3.0	**58**	20.0
7	0.4	**59**	> 100
8	0.4	**60**	16.0
9	3.5	**61**	3.0
10	17.0	**62**	3.5
***E* 11**	6.0	**63**	16.0
***Z* 11**	0.08	**64**	0.4
12	2.5	**65**	> 100
13	7.5	**66**	> 100
14	0.1	**67**	> 100
15	> 100	***E* 68**	> 100
16	89.0	***Z* 68**	80.0
***E* 17**	27.0	**69**	> 100
***Z* 17**	30.0	**70**	> 100
48	> 100	**71**	39.0
49	> 100	**72**	7.0
50	0.4	**73**	18
51	> 100	**75**	6.0
52	11.0	**76**	> 100
53	17.0	**77**	5.0
54	16.0	**78**	0.4
55	15.0		

[a] Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method. Data represent mean values for two separate experiments. Variation among duplicate samples was less than 15%.

the required substituted benzaldehyde (**3**) (8.35–13.86 mmol) in the same solvent (15 mL). After addition was complete, the whole mixture was heated under reflux for 20–72 h, as reported below. In one case, as indicated, a second mole equivalent of benzaldehyde-derivative was added after 24 h and the reflux continued for an additional 20 h. The desired compounds (only *E* isomers), unless otherwise specified, were obtained by filtration of the resulting precipitates as soon as the reaction mixture reaches r.t. Additional amounts of product were generally obtained by chromatography on silica gel column (eluent Et_2O–light petroleum 70:30) of the crude residue obtained after evaporation of the $C_6H_5CH_3$ solution. Analytical samples have been recrystallized from a suitable solvent, as reported below. Yields, reaction conditions, m.p.'s, analytical and spectroscopical data are reported as follows.

*6.2.1. E-2-(1H-benzotriazol-1-yl)-3-(2-fluorophenyl)acrylonitrile (**18**)*

This compound was obtained in 75% yield starting from **1** (1.2 g, 7.6 mmol) and 2-fluorobenzaldehyde (1.06 g, 8.50 mmol) after 20 h under reflux; m.p. 141–42 °C (from C_3H_6O); IR (Nujol): ν 2220 (CN), 1610, 1580, cm^{-1}; UV (EtOH): λ_{max} 321, 281, 206 nm; ^{1}H-NMR ($CDCl_3$): δ 8.16 (d, 1H, $J = 8.4$ Hz, H-4), 7.96 (s, 1H, vinyl-H), 7.95 (d, 1H, $J = 8.4$ Hz, H-7), 7.68 (m, 3H, H-6+2 phenyl H), 7.54 (m, 2H, H-5+1 phenyl H), 7.26 (dd, 1H, $J = 8.2$ Hz, 1 phenyl H); MS: *m/z* 264 [M^+]. Anal. $C_{15}H_9FN_4$ (C, H, N).

*6.2.2. E-2-(1H-benzotriazol-1-yl)-3-(2-chlorophenyl)acrylonitrile (**19**)*

This compound was obtained in 45% yield starting from **1** (2 g, 12.6 mmol) and 2-chlorobenzaldehyde (1.9 g, 17.6 mmol) heated for 24 h under reflux, then an extra portion of 2-chlorobenzaldehyde (1.9 g, 17.6 mmol) was

Table 4
Activity against hematological human tumor-derived cell lines of compounds (**5**, **7**, **8**, **Z 11**, **14**, **30**, **31**, **36**, **38**, **40**, **50**, **64**, and **78**)

Compound	IC_{50} [a]		
	CCRF-CCM [b]	WIL-2NS [c]	CCRF-SB [d]
5	0.2	0.2	0.07
7	0.9	1.8	0.9
8	0.4	0.6	0.3
***Z* 11**	0.6	0.8	0.6
14	0.9	0.6	0.6
30	1.2	6.6	4.9
31	1.3	1.8	1.7
34	0.2	0.1	0.09
36	2.1	5.8	6.2
38	3.2	5.7	6.1
40	3.0	9.5	9.0
50	1.0	2.0	1.0
64	1.4	1.0	0.6
78	0.4	0.9	0.3
6MP	1.0	3.1	1.1
Etoposide	0.09	0.2	0.1

[a] Compound concentration (μM) required to reduce cell proliferation by 50%, as determined by the MTT method, under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication. Data represent mean values for two separate experiments. Variation among duplicate samples was less than 15%.
[b] $CD4^+$ human acute T-lymphoblastic leukemia.
[c] Human splenic B-lymphoblastoid cells.
[d] Human acute B-lymphoblastic leukemia.

added and the reflux continued for an additional 20 h; m.p. 111–112 °C (from C_3H_6O); IR (Nujol): *v* 2230 (CN), 1620, 1600, 1580 cm^{-1}; UV (EtOH): λ_{max}: 319, 279, 237, 209 nm; ^{1}H-NMR ($CDCl_3$): δ 8.28 (s, 1H, vinyl-H), 8.17 (d, 1H, $J = 8.2$ Hz, H-4), 8.15 (d, 1H, $J =$ 2.4 Hz, H-3′), 7.95 (d, 1H, $J = 8.2$ Hz, H-7), 7.67 (dd, 1H, $J = 8.2$ and 2.4 Hz, H-6), 7.58–7.40 (m, 4H, H-5+3 phenyl H); MS: *m/z* 280/282 [M^+]. Anal. $C_{15}H_9ClN_4$ (C, H, Cl, N).

6.2.3. *E-2-(1H-benzotriazol-1-yl)-3-(2-bromophenyl)acrylonitrile (**20**)*

This compound was obtained in 72% yield starting from **1** (1.5 g, 9.5 mmol) and 2-bromobenzaldehyde (2.09 g, 11.3 mmol) after 22 h under reflux; m.p. 116–117 °C (from C_3H_6O); IR (Nujol): *v* 2220 (CN), 1600, 1580 cm^{-1}; UV (EtOH): λ_{max} 318, 279, 240, 206 nm; ^{1}H-NMR (Me_2CO-d_6): δ 8.27 (s, 1H, vinyl-H), 8.24–8.12 (m, 3H, H-4+H-7+H-3′), 7.88 (dd, 1H, $J = 8.0$ and 1.2 Hz, H-6), 7.80 (dd, 1H, $J = 8.0$ and 1.2 Hz, H-5), 7.64 (m, 3H, phenyl H); MS: *m/z* 324/326 [M^+]. Anal. $C_{15}H_9BrN_4$ (C, H, Br, N).

6.2.4. *E-2-(1H-benzotriazol-1-yl)-3-(2-iodophenyl)acrylonitrile (**21**)*

This compound was obtained in 59% yield starting from **1** (1 g, 6.3 mmol) and 2-iodobenzaldehyde (1.5 g,

Table 5
Activity against solid human tumor-derived cell lines of compounds (**5**, **7**, **8**, Z **11, 14**, **30**, **31**, **36**, **38**, **40**, **50**, **64**, and **78**)

Compound	IC_{50} [a]				
	SKMEL28 [b]	MCF7 [c]	SKMES-1 [d]	HepG2 [e]	DU145 [f]
5	0.3	0.7	1.7	2.2	0.5
7	2.3	1.8	4.7	3.3	2.6
8	1.1	0.7	2.4	2.1	1.7
***Z*·11**	1.6	1.2	1.6	10.4	1.2
14	3.3	1.3	3.3	n.d.	3.4
30	7.0	9.9	13.5	7.0	4.4
31	4.0	n.d.	4.6	3.4	2.4
34	0.2	0.1	0.6	0.8	0.6
36	2.8	5.5	4.2	6.0	3.3
38	6.7	n.d.	6.7	6.5	3.4
40	6.8	10.0	10.0	12.0	5.6
50	2.1	1.8	3.4	3.1	2.6
64	3.3	1.5	1.8	1.0	1.8
78	1.0	3.2	1.9	8.3	1.5
6MP	15	3.2	58.0	8.0	2.0
Etoposide	1.2	1.0	0.3	0.7	0.4

[a] Compound concentration (μM) required to reduce cell proliferation by 50%, as determined by the MTT method, under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication. Data represent mean values for two separate experiments. Variation among duplicate samples was less than 15%.
[b] Human skin melanoma.
[c] Human breast adenocarcinoma.
[d] Human lung squamous carcinoma.
[e] Human hepatocellular carcinoma.
[f] Human prostate carcinoma.

6.46 mmol) after 25 h under reflux; m.p. 137–138 °C (from $CHCl_3$); IR (Nujol): *v* 2220 (CN), 1610, 1570 cm^{-1}; UV (EtOH): λ_{max} 302, 259, 234, 207 nm; ^{1}H-NMR ($CDCl_3$): δ 8.18 (d, 1H, $J = 8.2$ Hz, H-4), 8.09 (s, 1H, vinyl-H), 8.07–7.95 (m, 2H, H-7+H-3′), 7.67 (dd, 1H, $J = 8.2$ and 1.2 Hz, H-6), 7.60–7.45 (m, 3H, phenyl H), 7.22 (dd, 1H, $J = 8.2$ and 1.2 Hz, H-5); MS: *m/z* 372 [M^+]. Anal. $C_{15}H_9IN_4$ (C, H, N).

6.2.5. *E-2-(1H-benzotriazol-1-yl)-3-(2-trifluoromethylphenyl)acrylonitrile (**22**)*

This compound was obtained in 50% yield starting from **1** (1.4 g, 8.85 mmol) and 2-trifluoromethylbenzaldehyde (1.85 g, 10.6 mmol) after 25 h under reflux; m.p. 109–110 °C (from C_3H_6O); IR (Nujol): *v* 2220 (CN), 1610, 1570 cm^{-1}; UV (EtOH): λ_{max} 314, 271, 205 nm; ^{1}H-NMR ($CDCl_3$): δ 8.30 (s, 1H, vinyl-H), 8.18 (d, 1H, $J = 8.2$ Hz, H-4), 8.12 (d, 1H, $J = 8.2$ Hz, H-7), 7.93 (dd, 1H, $J = 8.2$ and 1.2 Hz, H-6), 7.84–7.61 (m, 4H, phenyl H), 7.60–7.45 (m, 3H, 3 phenyl H), 7.52 (dd, 1H, $J = 8.2$ and 1.2 Hz, H-5); MS: *m/z* 314 [M^+]. Anal. $C_{16}H_9F_3N_4$ (C, H, N).

6.2.6. *E-2-(1H-benzotriazol-1-yl)-3-(2-nitrophenyl)acrylonitrile (**23**)*

This compound was obtained in 56% yield starting from **1** (2 g; 12.6 mmol) and 2-nitrobenzaldehyde (2.4 g; 25.2 mmol) after 72 h under reflux; m.p. 186–187 °C (from C_3H_6O); IR (Nujol): *v* 2220 (CN), 1610, 1590 cm^{-1}; UV (EtOH): λ_{max} 310, 260, 207, nm; ^{1}H-NMR ($CDCl_3$): δ 8.45 (s, 1H, vinyl-H), 8.37 (d, 1H, $J = 8.2$ Hz, H-3′), 8.19 (d, 1H, $J = 8.2$ Hz, H-4), 8.02 (d, 2H, $J = 8.2$ Hz, H-7+H-6′), 7.90 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-6), 7.81–7.65 (m, 2H, H-4′+H-5′) 7.53 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-5); MS: *m/z* 291 [M^+]. Anal. $C_{15}H_9N_5O_2$ (C, H, N).

6.2.7. *E-2-(1H-benzotriazol-1-yl)-3-(2-methoxyphenyl)acrylonitrile (**24**)*

This compound was obtained in 34% yield starting from **1** (1.5 g; 9.45 mmol) and 2-methoxybenzaldehyde (1.42 g; 10.4 mmol) after 20 h under reflux; m.p. 114–115 °C (from ether); IR (Nujol): 2220 (CN), 1610, 1560 cm^{-1}; UV (EtOH): λ_{max} 349, 314, 283, 237, 209 nm; ^{1}H-NMR ($CDCl_3$): δ 8.30 (s, 1H, vinyl-H), 8.18 (m, 2H, H-4+H-6′), 7.91 (d, 1H, $J = 8.2$ Hz, H-7), 7.67–7.45 (m, 3H, H-5+H-6+H-4′), 7.13 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-5′), 7.01 (d, 1H, $J = 8.2$ Hz, H-3′), 3.91 (s, 3H, CH_3); MS: *m/z* 276 [M^+]. Anal. $C_{16}H_{12}N_4O$ (C, H, N).

6.2.8. *E-2-(1H-benzotriazol-1-yl)-3-(3-fluorophenyl)acrylonitrile (**25**)*

This compound was obtained in 72.5% yield starting from **1** (1.2 g, 7.6 mmol) and 3-fluorobenzaldehyde (1.17 g, 9.43 mmol) after 20 h under reflux; m.p. 108–109 °C (from C_3H_6O); IR (Nujol): *v* 2230 (CN), 1600, 1580 cm^{-1}; UV (EtOH): λ_{max} 322, 279, 208 nm; ^{1}H NMR ($CDCl_3$): δ 8.26 (m, 2H, H-4+H-2′), 8.17 (m, 2H, vinyl-H+H-4′), 7.93 (d, 1H, $J = 8.2$ Hz, H-7), 7.70–7.47 (m, 2H, H-6+H-6′), 7.39–7.16 (m, 2H, H-5+H-5′); MS: *m/z* 264 [M^+]. Anal. $C_{15}H_9FN_4$ (C, H, N).

6.2.9. *E-2-(1H-benzotriazol-1-yl)-3-(3-chlorophenyl)acrylonitrile (**26**)*

This compound was obtained in 79% yield starting from **1** (2.5 g, 15.8 mmol) and 3-chlorobenzaldehyde (2.65 g, 15.9 mmol) after reflux for 20 h; m.p. 149–150 °C (from C_3H_6O); IR (Nujol): *v* 2220 (CN), 1590, 1570 cm^{-1}; UV (EtOH): λ_{max} 319, 281, 206 nm; ^{1}H-NMR ($CDCl_3$): δ 8.16 (d, 1H, $J = 8.2$ Hz, H-4), 7.95 (d, 1H, $J = 8.2$ Hz, H-7), 7.93 (s, 1H, vinyl-H), 7.87–7.83 (m, 2H, phenyl H), 7.66 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-6), 7.54–7.45 (m, 3H, H-5+phenyl H); MS: *m/z* 280/282 [M^+]. Anal. $C_{15}H_9ClN_4$ (C, H, Cl, N).

6.2.10. *E-2-(1H-benzotriazol-1-yl)-3-(3-bromophenyl)acrylonitrile (**27**)*

This compound was obtained in 62% yield from **1** (1.5 g, 9.5 mmol) and 3-bromobenzaldehyde (2.09 g, 11.3 mmol) after 22 h under reflux; m.p. 139–140 °C (from C_3H_6O); IR (Nujol): *v* 2220 (CN), 1600, 1590, 1570 cm^{-1}; UV (EtOH): λ_{max} 321, 282, 234, 209 nm; ^{1}H-NMR (Me_2CO-d_6): δ 8.24–8.07 (m, 3H, H-4+H-7+H-2′), 8.18 (s, 1H, vinyl-H), 7.83–7.72 (m, 2H, H-5+H-6), 7.65–7.50 (m, 3H, 3 phenyl H); MS: *m/z* 324/326 [M^+]. Anal. $C_{15}H_9BrN_4$ (C, H, Br, N).

6.2.11. *E-2-(1H-benzotriazol-1-yl)-3-(3-iodophenyl)acrylonitrile (**28**)*

This compound was obtained in 62% yield from **1** (1.4 g, 8.85 mmol) and 3-iodobenzaldehyde (2.2 g, 9.48 mmol) after 22 h under reflux; m.p. 134–135 °C (from $CHCl_3$); IR (Nujol): *v* 2210 (CN), 1610, 1580 cm^{-1}; UV (EtOH): λ_{max} 306, 268, 226, 212, 206 nm; ^{1}H-NMR ($CDCl_3$): δ 8.16 (d, 1H, $J = 2.2$ Hz, H-2′), 7.99–7.86 (m, 4H, H-4+H-7+2 phenyl H), 7.89 (s, 1H,vinyl-H), 7.66 (dd, 1H, $J = 8.2$ and 1.2 Hz, H-6), 7.51 (dd, 1H, $J = 8.2$ and 1.2 Hz, H-5), 7.29 (dd, 1H, $J = 8.0$ and 2.2 Hz, H-4′); MS: *m/z* 372 [M^+]. Anal. $C_{15}H_9IN_4$ (C, H, N).

6.2.12. *E-2-(1H-benzotriazol-1-yl)-3-(3-trifluoromethylphenyl)acrylonitrile (**29**)*

This compound was obtained in 58.5% yield starting from **1** (1.45 g, 9.2 mmol) and 3-trifluoromethylbenzaldehyde (1.76 g, 10.1 mmol) after 20 h under reflux; m.p. 106–107 °C (from C_3H_6O); IR (Nujol): *v* 2240 (CN), 1625, 1600, 1590, 1580 cm^{-1}; UV (EtOH): λ_{max} 320, 278, 205 nm; ^{1}H-NMR ($CDCl_3$): δ 8.18 (dd, 1H, $J = 7.6$ and 1.8 Hz, H-4′), 8.07 (d, 1H, $J = 8.2$ Hz, H-4), 8.04 (s, 1H, vinyl-H), 7.96 (d, 1H, $J = 8.4$ Hz, H-7), 7.79 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-6), 7.74 (m, 3H, 3 phenyl H),

7.52 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-5); MS: *m/z* 314 [M^+]. Anal. $C_{16}H_9F_3N_4$ (C, H, N).

*6.2.13. E-2-(1H-benzotriazol-1-yl)-3-(3-nitrophenyl)acrylonitrile (**30**)*

This compound was obtained in 60% yield from **1** (2 g; 12.6 mmol) and 3-nitrobenzaldehyde (1.90 g; 12.6 mmol) after 30 h under reflux; m.p. 177–178 °C (from C_3H_6O); IR (Nujol): *v* 2210 (CN), 1630, 1590, 1560 cm^{-1}; UV (EtOH): λ_{max} 321, 269, 204 nm; ^{1}H-NMR ($CDCl_3$): δ 8.71 (s, 1H, vinyl-H), 8.43–8.31 (m, 2H, H-4′+H-6′), 8.19 (d, 1H, $J = 8.2$ Hz, H-4), 8.13 (s, 1H, H-2′), 8.00 (d, 1H, $J = 8.2$ Hz, H-7), 7.78 (dd, 2H, $J = 8.2$ and 1.8 Hz, H-6), 7.70 (dd, 2H, $J = 8.2$ and 1.8 Hz, H-5′), 7.54 (dd, 2H, $J = 8.2$ and 1.8 Hz, H-5); MS: *m/z* 291 [M^+]. Anal. $C_{15}H_9N_5O_2$ (C, H, N).

*6.2.14. E-2-(2H-benzotriazol-1-yl)-3-(3-methoxyphenyl)acrylonitrile (**31**)*

This compound was obtained in 35% yield starting from **1** (1 g; 6.3 mmol) and 3-methoxybenzaldehyde (0.9 g; 6.5 mmol) after 22 h under reflux; m.p. 131–132 °C (from ether); IR (Nujol): 2240 (CN), 1615, 1570 cm^{-1}; UV (EtOH): λ_{max} 320, 287, 243, 213 nm; ^{1}H-NMR ($CDCl_3$): δ 8.16 (d, 1H, $J = 8.2$ Hz, H-4), 7.93 (d, 1H, $J = 8.2$ Hz, H-7), 7.91 (s, 1H, vinyl-H), 7.65 (dd, 2H, $J = 8.2$ and 1.8 Hz, H-6), 7.54–7.44 (m, 4H, H-5+H-7), (d, 1H, $J = 3.0$ Hz, H-5′), 7.71 (d, 1H, $J = 4.8$ Hz, H-3′), 7.48–7.43 (m, 2H, H-5+3 phenyl H), 7.09 (d, 1H, $J =$ 7.2 Hz, H-4′), 3.90 (s, 3H, CH_3); MS: *m/z* 276 [M^+]. Anal. $C_{16}H_{12}N_4O$ (C, H, N).

*6.2.15. E-2-(1H-benzotriazol-1-yl)-3-(3-cyanophenyl)acrylonitrile (**32**)*

This compound was obtained in 51% yield starting from **1** (1.4 g, 8.85 mmol) and 3-cyanobenzaldehyde 1.5 g, 11.4 mmol), after 24 h under reflux; m.p. 177–178 °C; IR (Nujol): *v* 2220 (CN), 1600, 1580 cm^{-1}; UV (EtOH): λ_{max} 321, 276, 208 nm; ^{1}H-NMR ($CDCl_3$): δ 8.23–8.12 (m, 3H, H-4+H-7+H-2′), 8.03 (s, 1H, vinyl-H), 7.98 (d, 1H, $J = 8.2$ Hz, H-4′), 7.83 (d, 1H, $J = 7.8$ Hz, H-6′), 7.74–7.64 (m, 2H, H-6+H-5′), 7.53 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-5); MS: *m/z* 271 [M^+]. Anal. $C_{16}H_9N_5$ (C, H, N).

*6.2.16. E-2-(1H-benzotriazol-1-yl)-3-(4-iodophenyl)acrylonitrile (**33**)*

This compound was obtained in 42% yield starting from **1** (1.2 g, 8.0 mmol) and 4-iodobenzaldehyde (1.88 g, 8.0 mmol) after 20 h under reflux; m.p. 139–140 °C (from C_3H_6O); IR (Nujol): *v* 2220 (CN), 1600, 1565 cm^{-1}; UV (EtOH): λ_{max} 330, 234, 206 nm; ^{1}H-NMR ($CDCl_3$): δ 8.15 (d, 1H, $J = 8.4$ Hz, H-4), 7.96–7.85 (m, 3H, H-7+H-3′+H-5′), 7.91 (s, 1H, vinyl-H), 7.69–7.61 (m, 3H, H-6+H-2′+H-6′), 7.50 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-5); MS: *m/z* 372 [M^+]. Anal. $C_{15}H_9IN_4$ (C, H, N).

*6.2.17. E-2-(1H-benzotriazol-1-yl)-3-(4-methoxyphenyl)acrylonitrile (**34**)*

This compound was obtained in 31% yield from **1** (1.50 g; 9.5 mmol) and 4-methoxybenzaldehyde (1.42 g; 10.4 mmol) after 24 h under reflux; m.p. 115–116 °C (from ether); IR (Nujol): *v* 2230 (CN), 1630, 1610, 1590 cm^{-1}; UV (EtOH): λ_{max} 330, 285, 240, 210 nm; ^{1}H-NMR ($CDCl_3$): δ 8.14 (d, 1H, $J = 8.4$ Hz, H-4), 7.95–7.84 (m, 3H, H-7+H-2′+H-6′), 7.91 (s, 1H, vinyl-H), 7.63 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-6), 7.48 (dd, 2H, $J = 8.4$ and 1.8 Hz, H-5), 7.05 (d, 2H, $J = 8.4$ Hz, H-3′+H-5′), 3.92 (s, 3H, CH_3); MS: *m/z* 276 [M^+]. Anal. $C_{16}H_{12}N_4O$ (C, H, N).

*6.2.18. E-2-(1H-benzotriazol-1-yl)-3-(4-cyanophenyl)acrylonitrile (**35**)*

This compound was obtained in 52.5% yield starting from **1** (1.4 g, 8.85 mmol) and 4-cyanobenzaldehyde (1.5 g, 11.4 mmol), after 20 h under reflux; m.p. 201–202 °C (from C_3H_6O); IR (Nujol): *v* 2230 (CN), 1600, 1580 cm^{-1}; UV (EtOH): λ_{max} 328, 281, 206 nm; ^{1}H-NMR ($CDCl_3$): δ 8.18 (d, 1H, $J = 8.2$ Hz, H-4), 8.06 (s, 1H, vinyl-H), 8.03 (d, 2H, $J = 8.2$ Hz, H-3′+H-5′), 7.99 (d, 1H, $J = 8.4$ Hz, H-7), 7.84 (d, 2H, $J = 8.4$ Hz, H-2′+H-6′), 7.69 (dd, 1H, $J = 8.2$ and 1.0 Hz, H-6), 7.53 (dd, 1H, $J = 8.2$ and 1.0 Hz, H-5); MS: *m/z* 271 [M^+]. Anal. $C_{16}H_9N_5$ (C, H, N).

*6.2.19. E-2-(1H-benzotriazol-1-yl)-3-(2,3-dichlorophenyl)acrylonitrile (**36**)*

This compound was obtained in 38% yield starting from **1** (2 g, 12.6 mmol) and 2,3-dichlorobenzaldehyde (2.8 g, 16.0 mmol), after 40 h under reflux; m.p. 160–161 °C (from C_3H_6O–Et_2O); IR (Nujol): *v* 2230 (CN), 1620 cm^{-1}; UV (EtOH): λ_{max} 318, 276, 240, 205 nm; ^{1}H-NMR ($CDCl_3$): δ 8.28 (s, 1H, vinyl-H); 8.19 (d, 1H, $J = 8.0$ Hz, H-4), 8.02 (d, 1H, $J = 8.0$ Hz, H-7), 7.97 (d, 1H, $J = 8.3$ Hz, H-6′), 7.73–7.64 (m, 2H, H-6+H-4′), 7.53 (t, 1H, $J = 8.0$ Hz, H-5), 7.43 (t, 1H, $J = 8.0$ Hz, H-5′), MS: *m/z* 314/316/318 [M^+]. Anal. $C_{15}H_8Cl_2N_4$ (C, H, Cl, N).

*6.2.20. E-2-(1H-benzotriazol-1-yl)-3-(2,4-dichlorophenyl)acrylonitrile (**37**)*

This compound was obtained in 25% yield starting from **1** (2 g, 12.6 mmol) and 2,4-dichlorobenzaldehyde (2.8 g, 16.0 mmol), after 20 h under reflux; m.p. 161–162 °C (from C_3H_6O–Et_2O); IR (Nujol): *v* 2220 (CN), 1620 cm^{-1}; UV (EtOH): λ_{max} 325, 282, 240, 205 nm; ^{1}H-NMR ($CDCl_3$): δ 8.23 (s, 1H, vinyl-H); 8.19 (d, 1H, $J = 8.4$ Hz, H-4), 8.13 (d, 1H, $J = 8.4$ Hz, H-7), 7.95 (d, 1H, $J = 8.4$ Hz, H-6′), 7.68 (t, 1H, $J = 8.4$ Hz, H-6), 7.58 (d, 1H, $J = 2.0$ Hz, H-3′), 7.52 (t, 1H, $J = 8.4$ Hz, H-5),

7.47 (dd, 1H, $J = 8.4$ and 2.0 Hz, H-5′), MS: *m/z* 314/316/318 [M^+]. Anal. $C_{15}H_8Cl_2N_4$ (C, H, Cl, N).

*6.2.21. E-2-(1H-benzotriazol-1-yl)-3-(2,6-dichlorophenyl)acrylonitrile (**38**)*

This compound was obtained in 49% yield starting from **1** (2 g, 12.6 mmol) and 2,6-dichlorobenzaldehyde 2.8 g, 16.0 mmol), after 22 h under reflux; m.p. 136–137 °C (from Et_2O); IR (Nujol): *v* 2220 (CN), 1620 cm^{-1}; UV (EtOH): λ_{max} 304, 268, 205 nm; ^{1}H-NMR ($CDCl_3$): δ 8.19 (d, 1H, $J = 8.4$ Hz, H-4), 8.01 (d, 1H, $J = 8.4$ Hz, H-7), 7.94 (s, 1H, vinyl-H), 7.69 (t, 1H, $J =$ 8.4 Hz, H-6), 7.53 (t, 1H, $J = 8.4$ Hz, H-5), 7.50 (d, 2H, $J = 8.0$ Hz, H-3′+H-5′), 7.41 (t, 1H, $J = 8.0$ Hz, H-4′), MS: *m/z* 314/316/318 [M^+]. Anal. $C_{15}H_8Cl_2N_4$ (C, H, Cl, N).

*6.2.22. E-2-(1H-benzotriazol-1-yl)-3-(3,4-dichlorophenyl)acrylonitrile (**39**)*

This compound was obtained in 43% yield starting from **1** (2 g, 12.6 mmol) and 3,4-dichlorobenzaldehyde (2.8 g, 16.0 mmol), after 24 h under reflux; m.p. 187–188 °C (from Et_2O); IR (nujol): *v* 2220 (CN), 1590 cm^{-1}; UV (EtOH): λ_{max} 325, 285, 236, 205 nm; ^{1}H-NMR ($CDCl_3$): δ 8.18 (d, 1H, $J = 8.0$ Hz, H-4), 7.96 (d, 1H, $J = 8.0$ Hz, H-7), 7.94 (d, 1H, $J = 8.4$ Hz, H-6′), 7.92 (s, 1H, vinyl-H), 7.83 (dd, 2H, $J = 8.4$ and 2.0 Hz, H-5′), 7.63 (t, 1H, $J = 8.4$ Hz, H-6), 7.66 (d, 1H, $J = 2.0$ Hz, H-2′), 7.52 (t, 1H, $J = 8.4$ Hz, H-5), MS: *m/z* 314/316/318 [M^+]. Anal. $C_{15}H_8Cl_2N_4$ (C, H, Cl, N).

*6.2.23. E-2-(1H-benzotriazol-1-yl)-3-(3,5-dichlorophenyl)acrylonitrile (**40**)*

This compound was obtained in 51% yield starting from **1** (2 g, 12.6 mmol) and 3,5-dichlorobenzaldehyde (2.8 g, 16.0 mmol), after 32 h under reflux; m.p. 196–197 °C (from $CHCl_3$–C_6H_{14}); IR (Nujol): *v* 2220 (CN), 1620 cm^{-1}; UV (EtOH): λ_{max} 322, 280, 205 nm; ^{1}H-NMR ($CDCl_3$): δ 8.17 (d, 1H, $J = 8.4$ Hz, H-4), 7.96 (d, 1H, $J = 8.4$ Hz, H-7), 7.91 (s, 1H, vinyl-H), 7.77 (d, 2H, $J = 2.0$ Hz, H-2′+H-6′), 7.68 (t, 1H, $J = 8.4$ Hz, H-6), 7.55–7.48 (m, 2H, H-5+H-4′), MS: *m/z* 314/316/318 [M^+]. Anal. $C_{15}H_8Cl_2N_4$ (C, H, Cl, N).

*6.2.24. E-2-(1H-benzotriazol-1-yl)-3-(2,3-difluorophenyl)acrylonitrile (**41**)*

This compound was obtained in 38% yield starting from **1** (2 g, 12.6 mmol) and 2,3-difluorobenzaldehyde (2.0 g, 16.0 mmol), after 30 h under reflux; m.p. 126–127 °C (from Et_2O); IR (Nujol): *v* 2230 (CN), 1625, 1580 cm^{-1}; UV (EtOH): λ_{max} 318, 276, 205 nm; ^{1}H-NMR ($CDCl_3$): δ 8.19 (d, 1H, $J = 8.2$ Hz, H-4), 8.17 (s, 1H, vinyl-H), 8.03 (d, 1H, $J = 8.2$ Hz, H-7), 7.95 (d, 1H, $J = 8.4$ Hz, H-6′), 7.68 (t, 1H, $J = 8.2$ Hz, H-6), 7.52 (t, 1H, $J = 8.2$ Hz, H-5), 7.40–7.29 (m, 2H, H-4′+H-5′), MS: *m/z* 282 [M^+]. Anal. $C_{15}H_8F_2N_4$ (C, H, N).

*6.2.25. E-2-(1H-benzotriazol-1-yl)-3-(2,4-difluorophenyl)acrylonitrile (**42**)*

This compound was obtained in 32% yield starting from **1** (2 g, 12.6 mmol) and 2,4-difluorobenzaldehyde (2.0 g, 16.0 mmol), after 22 h under reflux; m.p. 107–108 °C (from Et_2O); IR (Nujol): *v* 2220 (CN), 1610 cm^{-1}; UV (EtOH): λ_{max} 320, 278, 205 nm; ^{1}H-NMR ($CDCl_3$): δ 8.30 (m, 1H, H-6′), 8.17 (d, 1H, $J = 8.2$ Hz, H-4), 8.11 (s, 1H, vinyl-H), 7.93 (d, 1H, $J = 8.2$ Hz, H-7), 7.66 (t, 1H, $J = 8.2$ Hz, H-6), 7.51 (t, 1H, $J = 8.2$ Hz, H-5), 7.14–6.94 (m, 2H, H-3′+H-5′), MS: *m/z* 282 [M^+]. Anal. $C_{15}H_8F_2N_4$ (C, H, N).

*6.2.26. E-2-(1H-benzotriazol-1-yl)-3-(2,6-difluorophenyl)acrylonitrile (**43**)*

This compound was obtained in 34% yield starting from **1** (2 g, 12.6 mmol) and 2,6-difluorobenzaldehyde (2.0 g, 16.0 mmol), after 23 h under reflux; m.p. 167–168 °C (from Et_2O); IR (Nujol): *v* 2240 (CN), 1620 cm^{-1}; UV (EtOH): λ_{max} 312, 274, 205 nm; ^{1}H-NMR ($CDCl_3$): δ 8.18 (d, 1H, $J = 8.4$ Hz, H-4), 8.00 (d, 1H, $J = 8.4$ Hz, H-7), 7.92 (s, 1H, vinyl-H), 7.68 (t, 1H, $J =$ 8.4 Hz, H-6), 7.59–7.42 (m, 2H, H-5+H-4′), 7.15–7.04 (m, 2H, H-3′+H-5′), MS: *m/z* 282 [M^+]. Anal. $C_{15}H_8F_2N_4$ (C, H, N).

*6.2.27. E-2-(1H-benzotriazol-1-yl)-3-(3,4-difluorophenyl)acrylonitrile (**44**)*

This compound was obtained in 35% yield starting from **1** (2 g, 12.6 mmol) and 3,4–difluorobenzaldehyde (2.0 g, 16.0 mmol), after 23 h under reflux; m.p. 142–143 °C (from Et_2O); IR (Nujol): *v* 2220 (CN), 1610 cm^{-1}; UV (EtOH): λ_{max} 320, 280, 205 nm; ^{1}H-NMR ($CDCl_3$): δ 8.17 (d, 1H, $J = 8.4$ Hz, H-4), 7.94 (d, 1H, $J = 8.4$ Hz, H-7), 7.92 (s, 1H, vinyl-H), 7.88–7.63 (m, 3H, H-6+H-2′+H-6′), 7.52 (t, 1H, H-5), 7.35 (dd, 1H, $J = 9.2$ and 8.6 Hz, H-5′), MS: *m/z* 282 [M^+]. Anal. $C_{15}H_8F_2N_4$ (C, H, N).

*6.2.28. E-2-(1H-benzotriazol-1-yl)-3-(3,5-difluorophenyl)acrylonitrile (**45**)*

This compound was obtained in 51% yield starting from **1** (2 g, 12.6 mmol) and 3,5-difluorobenzaldehyde (2.0 g, 16.0 mmol), after 22 h under reflux; m.p. 165–166 °C (from Et_2O); IR (Nujol): *v* 2230 (CN), 1620, 1590 cm^{-1}; UV (EtOH): λ_{max} 320, 278, 205 nm; ^{1}H-NMR ($CDCl_3$): δ 8.17 (d, 1H, $J = 8.2$ Hz, H-4), 7.97 (d, 1H, $J = 8.2$ Hz, H-7), 7.95 (s, 1H, vinyl-H), 7.68 (t, 1H, $J = 8.4$ Hz, H-6), 7.56–7.41 (m, 3H, H-5+H-2′+H-6′), 7.04–6.95 (m, 1H, H-4′), MS: *m/z* 282 [M^+]. Anal. $C_{15}H_8F_2N_4$ (C, H, N).

*6.2.29. E-2-(1H-benzotriazol-1-yl)-3-[2,4-bis(trifluoromethyl)phenyl]acrylonitrile (**46**)*

This compound was obtained in 32% yield starting from **1** (2 g, 12.6 mmol) and 2,4-bis(trifluoromethyl)ben-

zaldehyde (3.7 g, 15.6 mmol), after 22 h under reflux; m.p. 108–109 °C (from Et_2O); IR (Nujol): ν 2240 (CN), 1620, 1600 cm^{-1}; UV (EtOH): λ_{max} 316, 266, 205 nm; ^{1}H-NMR ($CDCl_3$): δ 8.33 (s, 1H, H-3′), 8.23 (d, 1H, $J = 8.2$ Hz, H-4), 8.21 (d, 1H, $J = 8.4$ Hz, H-5′), 8.10 (s, 1H, vinyl-H), 8.04 (d, 1H, $J = 8.2$ Hz, H-7), 7.97 (t, 1H, $J = 8.4$ Hz, H-6′), 7.71 (t, 1H, $J = 8.2$ Hz, H-6), 7.55 (t, 1H, $J = 8.2$ Hz, H-5), MS: m/z 382 [M^+]. Anal. $C_{17}H_8F_6N_4$ (C, H, N).

6.2.30. E-2-(1H-benzotriazol-1-yl)-3-[3,5-bis(trifluoromethyl)phenyl]acrylonitrile (**47**)

This compound was obtained in 44% yield starting from **1** (2 g, 12.6 mmol) and 3,5-bis(trifluoromethyl)benzaldehyde (3.7 g, 15.6 mmol), after 22 h under reflux; m.p. 130–131 °C (from Et_2O); IR (Nujol): ν 2230 (CN), 1620 cm^{-1}; UV (EtOH): λ_{max} 320, 272, 205 nm; ^{1}H-NMR ($CDCl_3$): δ 8.35 (s, 2H, H-2′ + H-6′), 8.19 (d, 1H, $J = 8.2$ Hz, H-4), 8.14 (s, 1H, vinyl-H), 8.03 (s, 1H, H-4′), 8.01 (d, 1H, $J = 8.2$ Hz, H-7), 7.70 (t, 1H, $J = 8.2$ Hz, H-6), 7.54 (t, 1H, $J = 8.2$ Hz, H-5), MS: m/z 382 [M^+]. Anal. $C_{17}H_8F_6N_4$ (C, H, N).

6.3. Microbiological assays

6.3.1. Compounds

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

6.3.2. Cells

Cell lines were purchased from American Type Culture Collection (ATCC). Hematological tumor-derived cells were grown in RPMI-1640 medium supplemented with 10% FCS, 100 units mL^{-1} penicillin G and 100 μg mL^{-1} streptomycin. Solid tumor-derived cells were grown in their specific media supplemented with 10% FCS and antibiotics. Cell cultures were incubated at 37 °C in a humidified, 5% CO_2 atmosphere. The absence of mycoplasma contamination was checked periodically by the Hoechst staining method.

6.3.3. Antiproliferative assays

Exponentially growing cells were resuspended in growth medium containing serial dilutions of the drugs. Cell viability was determined after 96 h at 37 °C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [10].

6.3.4. Virus

Human immunodeficiency virus type 1 (HIV-1) was obtained from supernatants of persistently infected H9/III_B cells. The HIV-1 stock solution had a titre of 1.0×10^7 50% cell culture infectious dose ($CCID_{50}$)/mL.

6.3.4.1. Antiviral assays. Activity of compounds against HIV-1 was based on inhibition of virus-induced cytopathogenicity in MT-4 cells acutely infected at a multiplicity of infection of 0.01.

6.3.5. Antibacterial and antimycotic assays

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

6.3.6. Anti-mycobacterial assays

M. tuberculosis 27294 and *M. smegmatis* 19420 were ATCC strains, *M. fortuitum* was clinical isolate. MICs were assessed in microtiter plates by adding 20 μL aliquots of a culture suspension to 80 μL of Middlebrook 7H9 medium containing serial dilutions of test compounds. At the end of incubation, the number of viable mycobacteria was determined by the MTT method [10].

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