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Synthesis and antiproliferative activity of 3-aryl-2-[1*H*(2*H*)-benzotriazol-1(2)-yl]acrylonitriles variously substituted: Part 4

Antonio Carta [a,*], Michele Palomba [a], Gianpiero Boatto [a], Bernardetta Busonera [b], Marta Murreddu [b], Roberta Loddo [b]

[a] *Dipartimento Farmaco Chimico Tossicologico, Universita degli Studi di Sassari, via Muroni 23/a, 07100 Sassari, Italy*
[b] *Dipartimento di Scienze e Tecnologie Biomediche, Sezione di Microbiologia e Virologia Generale e Biotecnologie Microbiche, Università degli Studi di Cagliari, Cittadella Universitaria, 09042 Monserrato (Cagliari), Italy*

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Abstract

A new series of variously substituted 3-aryl-2-[1*H*(2*H*)-benzotriazol-1(2)-yl]acrylonitriles was synthesized and tested for antiproliferative and antitubercular activity as part of our continuing research program in the antimicrobial and antitumor fields. The most cytotoxic derivatives (**5a,g,i,j,l** and **7b**) (CC_{50} < 3.0 μM against MT-4 cells) were evaluated against a panel of human cell lines derived from hematological and solid tumors, using 6-mercaptopurine (6-MP) and etoposide as reference drugs. In particular, *E*-2-(5,6-dimethyl-1*H*-benzotriazol-1-yl)-3-(3-nitrophenyl)acrylonitrile (**5g**) resulted more potent than 6-MP on all cell lines, even if 2–14-fold less potent than etoposide. In the antitubercular screening, the derivatives **5i,j** and **7e** showed moderate activity against some resistant strains of *Mycobacterium* tested.

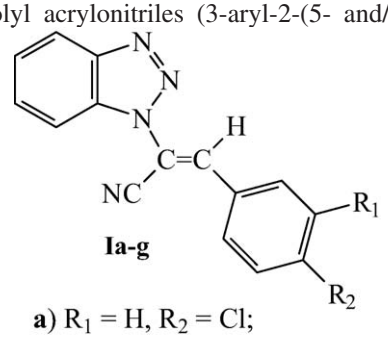
Keywords: 3-Aryl-2-(1*H*(2*H*)-benzotriazol-1(2)-yl)acrylonitriles; Antiproliferative activity; Antitubercular screening; SAR

1. Introduction

Recently, we reported the synthesis and antimycobacterial and antiproliferative activities of over 80 3-aryl-2-[1*H*(2*H*)-benzotriazol-1(2)-yl]acrylonitriles, prop-2-enamides and propenoic acids variously substituted in the aryl moiety [1–3]. In particular 3-aryl-2-(1*H*-benzotriazol-1-yl)acrylonitriles (**Ia–g**), summarized in Fig. 1, resulted active against both hematological and solid human tumors [3].

Furthermore, several compounds exhibited an interesting antitubercular activity, even if, in general, were cytotoxic against MT-4 cells at lower concentrations than those recorded against *Mycobacterium tuberculosis*. In any case we could observe that the cytotoxicity of (benzotriazol-2-yl)acrylonitriles was in general lower than that of (benzotriazol-1-yl)acrylonitriles [1–3].

On the basis of these considerations, and in line with our antimicrobial and antitumor research programs [4–9], we have now planned the preparation of two new series of benzotriazolyl acrylonitriles (3-aryl-2-(5- and/or 6-substi-



Ia-g

a) R_1 = H, R_2 = Cl;
b) R_1 = H, R_2 = Br;
c) R_1 = H, R_2 = CH_3;
d) R_1 = H, R_2 = OCH_3;
e) R_1 = NO_2, R_2 = H;
f) R_1 = OCH_3, R_2 = H;
g) R_1-R_2 = OCH_2O.

Fig. 1. 3-Aryl-2-(1*H*-benzotriazol-1-yl)acrylonitriles endowed with antiproliferative activity.

* Corresponding author.
E-mail address: acarta@ssmain.uniss.it (A. Carta).

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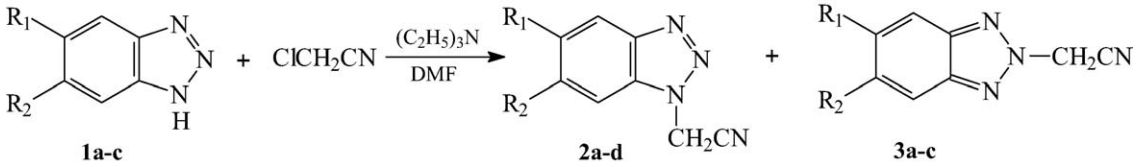
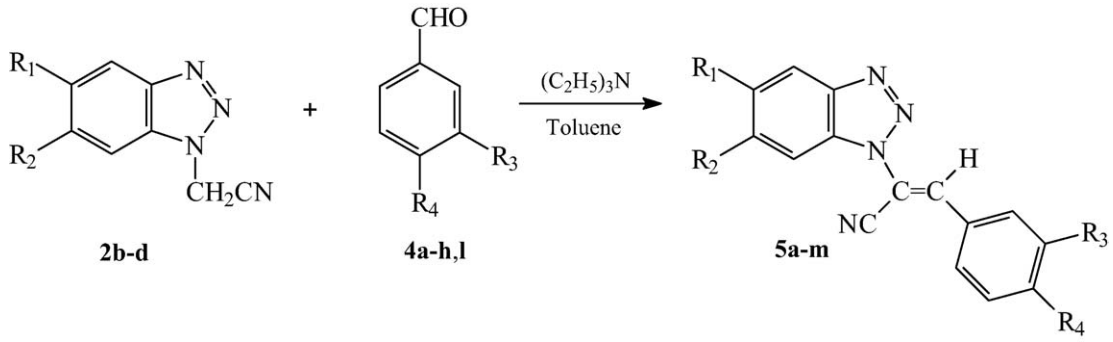
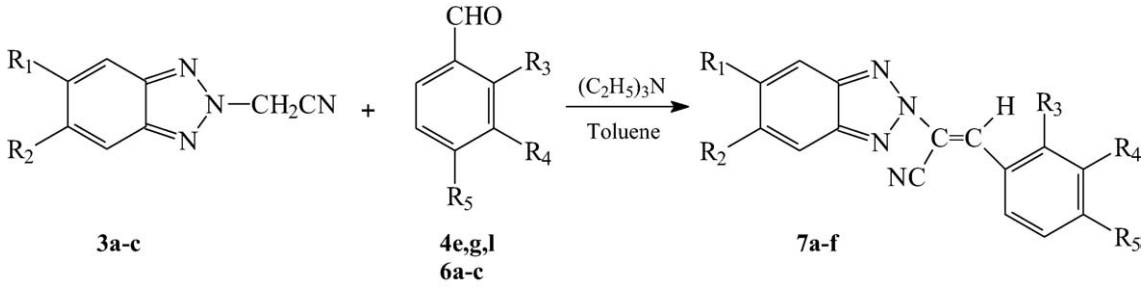


Scheme 1. Preparation of the intermediates **2a–d** and **3a–c**.



Scheme 2. Preparation of the final products **5a–m**.



Scheme 3. Preparation of the final products **7a–f**.

tuted-1*H*-benzotriazol-1-yl)acrylonitriles (**5a–m**) of Scheme 2 and Table 2 and 3-aryl-2-(5- and/or 6-substituted-2*H*-benzotriazol-2-yl)acrylonitriles (**7a–f**) of Scheme 3 and Table 3), in order to extend our previous structure-activity relationship (SAR) studies. The derivatives of the series **5** bear a trifluoromethyl or two methyl groups in the benzo-moiety in order to evaluate the effect of electron-releasing or electron-withdrawing groups associated with the increase of lipophilicity and steric hindrance on the antiproliferative activity. The substituents in the phenyl-moiety were those that showed the most antiproliferative activity in the previous series (Cl, Br, CH_3, NO_2, CF_3, OCH_3 and methylenedioxy).

Table 1
Substituents in the intermediates of Scheme 1 (**2a–d** and **3a–c**) and corresponding benzotriazoles (**1a–c**)

	R_1	R_2
a	H	H
b	CH_3	CH_3
c	CF_3	H
d	H	CF_3

The compounds of series **7** maintain the same feature in the benzo-moiety of the heterocycle, while in the side chain the phenyl-moiety was substituted using the most interesting groups found active against *M. tuberculosis* in the above-mentioned series like I, F, NO_2, OCH_3 and methylenedioxy [1–3].

Table 2
Substituents in the final products of Scheme 2 (**5a–m**) and corresponding aldehydes (**4a–h,l**)

	R_1	R_2	R_3	R_4
a	CH_3	CH_3	H	H
b	CH_3	CH_3	H	Cl
c	CH_3	CH_3	H	Br
d	CH_3	CH_3	H	CH_3
e	CH_3	CH_3	H	OCH_3
f	CH_3	CH_3	H	CF_3
g	CH_3	CH_3	NO_2	H
h	CH_3	CH_3	CF_3	H
i	CF_3	H	NO_2	H
j	H	CF_3	NO_2	H
k	CF_3	H	H	OCH_3
l	CH_3	CH_3	OCH_2O	
m	CF_3	H	OCH_2O	

Table 3
Substituents in the final products of Scheme 3 (**7a–f**) and corresponding aldehydes (**6a–c**)

	R_1	R_2	R_3	R_4	R_5
a	H	H	I	H	H
b	H	H	H	F	H
c	CH_3	CH_3	H	OCH_3	OCH_3
d	H	CF_3	H	H	OCH_3
e	H	CF_3	H	NO_2	H
f	H	CF_3	H	OCH_2O	

2. Chemistry

The intermediates benzotriazolyl acetonitriles (**2a–d** and **3a–c**) were prepared as described in Scheme 1 and Table 1 following the procedure previously reported for the known **2a** and **3a** [1,10], by condensation of the chloroacetonitrile with the opportune benzotriazole derivative **1a–c**, in dimethylformamide (DMF) in the presence of triethylamine. From the reaction mixture we obtained a mixture of isomers alkylated in position 1–3 which were separated by flash column chromatography on silica gel, where the *N*-2 substituted isomers eluted first. The exact assignment of their structure was easy determined by ^{1}H NMR spectroscopy in the case of 5,6-dimethyl derivatives (**2b** and **3b**) because of symmetry, while in the case of (**2c,d** and **3c**) it was necessary to run nuclear overhauser effect (NOE) experiments to identify unambiguously the single isolated isomers. Thus we have observed that the irradiation of CH_2 signal at δ 5.67 of the compounds **2c** caused a NOE between this and the doublet located at δ 7.82 of the H-7, thus confirming the position of alkylation. Similarly, irradiation at δ 5.59 caused a NOE between this and the singlet of the H-7 at δ 8.01 confirming structure **2d**. In the case of **3b**, as expected, no NOE was observed between the protons of the CH_2 group and the distant H-4 or H-7 protons. These data were completely in agreement with our previous study on similar derivatives [11,12]. The syntheses of the new series of 3-aryl-2-(1*H*-benzotriazol-1-yl)acrylonitriles **5a–m**, and 3-aryl-2-(2*H*-benzotriazol-2-yl)acrylonitriles **7a–f**, are described in Schemes 2 and 3 and Tables 2 and 3, respectively. These were accomplished as previously reported [1] by Knoevenagel condensation of intermediates (**2a–d** and **3a–c**) with the appropriate aldehydes (**4a–h,l** and **6a–c**), in toluene at reflux in the presence of triethylamine. The reactions of 2-(6-trifluoromethyl-1*H*-benzotriazol-1-yl)acetonitrile (**2d**), respectively, with 4-methoxybenzaldehyde (**4e**) and with (methylenedioxy)benzaldehyde (**4l**) failed, in spite of our attempts to modify reaction times and concentrations. This behavior is probably due at the steric hindrance of **2d** associated at the low electrophilicity of the carbon atom of the carbonyl of compounds **4e** and **4l**. In these new series we have isolated only *E*-isomers, even if in some cases (compounds **5j,k,m** and **7a,c,e,f**) not isolable traces of *Z*-isomers were identified by GC/MS.

^{1}H NMR and analytical (elemental analyses, MS) data of all the new compounds are in accordance with those of the previously described counterparts [1–3] and support the assigned chemical structure.

3. Experimental

Melting points were determined by a Kofler hot stage or Digital Electrothermal apparatus, and are uncorrected. ^{1}H NMR spectra were recorded on a Varian XL-200 (200 MHz) instrument, using tetramethylsilane (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 t (triplet). 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). The progress of the reactions was monitored by TLC using Merck F-254 commercial plates. Light petroleum refers to the fraction with b.p. 40–60 °C. The analytical results for C, H, N were within ±0.4% of the theoretical values.

3.1. Intermediates

Benzotriazole (**1a**), 5,6-dimethylbenzotriazole (**1b**) and the aldehydes (**4a–h,l** and **6b,c**) were commercially available, 5-trifluomethylbenzotriazole (**1c**) and 2-iodobenzaldehyde (**6a**) were prepared following the procedure previously described in Refs. [13,14].

3.1.1. Preparation of benzotriazolyl acetonitriles (*2a–d* and *3a–c*)

To a stirred solution of **1a–c** (50 mmol) and triethylamine (5.6 g, 55 mmol) in DMF (50–70 ml), chloroacetonitrile (5.7 g, 75 mmol) was slowly added dropwise. After the addition was complete, the reaction mixture was heated at 110 °C (for **1a,b**) or at 70 °C (for **1c**) then the stirring was continued for an additional 12 h. On cooling to room temperature (r.t.), the resulting precipitate of triethylamine hydrochloride was filtered off and the filtrate evaporated to dryness under reduced pressure. The resulting residue was flash chromatographed on silica gel (eluent: diethyl ether/light petroleum, 8:2) affording in sequence: 2-(2*H*-benzotriazol-2-yl)acetonitrile **3a** and 2-(1*H*-benzotriazol-1-yl)acetonitrile **2a** (in the case of the reaction of **1a**); 2-(5,6-dimethyl-2*H*-benzotriazol-2-yl)acetonitrile **3b** and 2-(5,6-dimethyl-1*H*-benzotriazol-1-yl)acetonitrile **2b** (in the case of the reaction of **1b**); 2-(5-trifluoromethyl-2*H*-benzotriazol-2-yl)acetonitrile **3c**, 2-(6-trifluoromethyl-1*H*-benzotriazol-1-yl)acetonitrile **2d**, and 2-(5-trifluoromethyl-1*H*-benzotriazol-1-yl)acetonitrile **2c** (in the case of the reaction of **1c**).

3.1.1.1. E-*2-(1*H*-benzotriazol-1-yl)acetonitrile (**2a**).* This compound was obtained in 73% yield; m.p. 86–87 °C (87 °C [10]).

3.1.1.2. E-*2-(2*H-*benzotriazol-2-yl)acetonitrile (**3a**).* This compound was obtained in 21% yield; m.p. 77–78 °C (78 °C [10]).

3.1.1.3. E-*2-(5,6-dimethyl-1*H-*benzotriazol-1-yl)acetonitrile (**2b**).* This compound was obtained in 42% yield; m.p. 111–113 °C (acetone); ^{1}H NMR ($CDCl_3$): δ 7.85 (s, 1H, H-4), 7.41 (s, 1H, H-7), 5.54 (s, 2H, CH_2), 2.47 (s, 3H, CH_3), 2.43 (s, 3H, CH_3); MS: *m/z* 186 (M^+). Anal. $C_{10}H_{10}N_4$ (C, H, N).

3.1.1.4. E-*2-(5,6-dimethyl-2*H-*benzotriazol-2-yl)acetonitrile (**3b**).* This compound was obtained in 13% yield; m.p. 121–123 °C (chloroform); ^{1}H NMR ($CDCl_3$): δ 7.59 (s, 2H, H-4 + H-7), 5.60 (s, 2H, CH_2), 2.40 (s, 6H, $2CH_3$); MS: *m/z* 186 (M^+). Anal. $C_{10}H_{10}N_4$ (C, H, N).

3.1.1.5. E-*2-(5-trifluoromethyl-1*H-*benzotriazol-1-yl)acetonitrile (**2c**).* This compound was obtained in 38% yield; m.p. 120–121 °C (diethyl ether); ^{1}H NMR ($CDCl_3$): δ 8.47 (s, 1H, H-4), 7.89 (d, 1H, J = 8.8 Hz, H-6), 7.82 (d, 1H, J = 8.8 Hz, H-7), 5.67 (s, 2H, CH_2); MS: *m/z* 226 (M^+). Anal. $C_9H_5F_3N_4$ (C, H, N).

3.1.1.6. E-*2-(6-trifluoromethyl-1*H-*benzotriazol-1-yl)acetonitrile (**2d**).* This compound was obtained in 26% yield; m.p. 101–102 °C (diethyl ether); ^{1}H NMR ($CDCl_3$): δ 8.28 (d, 1H, J = 8.8 Hz, H-4), 8.01 (s, 1H, H-7), 7.74 (d, 1H, J = 8.8 Hz, H-6), 5.69 (s, 2H, CH_2); MS: *m/z* 226 (M^+). Anal. $C_9H_5F_3N_4$ (C, H, N).

3.1.1.7. E-*2-(5-trifluoromethyl-2*H-*benzotriazol-2-yl)acetonitrile (**3c**).* This compound was obtained in 20% yield; m.p. 68–69 °C (diethyl ether); ^{1}H NMR ($CDCl_3$): δ 8.26 (d, 1H, J = 1.4 Hz, H-4), 8.04 (d, 1H, J = 8.8 Hz, H-7), 7.65 (dd, 1H, J = 8.8 and 1.4 Hz, H-6), 5.71 (s, 2H, CH_2); MS: *m/z* 226 (M^+). Anal. $C_9H_5F_3N_4$ (C, H, N).

3.2. General procedure for preparation of E-*2-(1*H-*benzotriazol-1-yl)-3-arylacrylonitriles (**5a–m**) and* E-*2-(2*H-*benzotriazol-2-yl)-3-arylacrylonitriles (**7a–f**)*

A solution of the appropriate 2-(1*H*-benzotriazol-1-yl)-acetonitrile (**2b–d**) or 2-(2*H*-benzotriazol-2-yl)-acetonitrile (**3a–c**) (6.0 mmol) and triethylamine (15.0 mmol) in toluene (20 ml) was stirred at r.t. for 10 min and added of a solution of the required substituted benzaldehyde (**4a–i,j,k,m** and **6a–f**) (7.2 mmol) in the same solvent (10 ml). After the addition was complete, the mixture was heated under reflux for 16–120 h, as reported below. In a few cases (**5a,m** and **7b**), as indicated, a second identical amount of benzaldehyde derivative was added after 24 h and the reflux continued for an additional 4–72 h. After removal of the solvent, the solid or oil crude residue was purified by chromatography on silica gel column (eluent: mixture of diethyl ether/hexane in 60:40 ratio).

Analytical samples have been recrystallized from a suitable solvent, as reported below. Yields, reaction times, m.p., MS and ^{1}H NMR data are reported as follows.

3.2.1. E-*2-(5,6-dimethyl-1*H-*benzotriazol-1-yl)-3-phenylacrylonitrile (**5a**)*

This compound was obtained in 36% yield starting from **2b** and benzaldehyde (**4a**) heated for 24 h under reflux, then an extra portion of **4a** was added and the reflux continued for an additional 72 h; m.p. 106–107 °C (acetone); ^{1}H NMR ($CDCl_3$): δ 7.98–7.91 (m, 3H, H-2′ + H-6′ + vinyl-H), 7.87 (s, 1H, H-4), 7.67 (s, 1H, H-7), 7.56–7.53 (m, 3H, H-3′ + H-4′ + H-5′), 2.48 (s, 3H, CH_3), 2.45 (s, 3H, CH_3); MS: *m/z* 274 (M^+). Anal. $C_{17}H_{14}N_4$ (C, H, N).

3.2.2. E-*2-(5,6-dimethyl-1*H-*benzotriazol-1-yl)-3-(4-chlorophenyl)acrylonitrile (**5b**)*

This compound was obtained in 45% yield starting from **2b** and 4-chlorobenzaldehyde (**4b**) after 24 h under reflux, m.p. 162–164 °C (acetone); ^{1}H NMR ($CDCl_3$): δ 7.92–7.90 (m, 3H, H-2′ + H-6′ + vinyl-H), 7.87 (s, 1H, H-4), 7.67 (s, 1H, H-7), 7.49–7.52 (m, 2H, H-3′ + H-5′), 2.48 (s, 3H, CH_3), 2.44 (s, 3H, CH_3); MS: *m/z* 308/310 (M^+). Anal. $C_{17}H_{13}ClN_4$ (C, H, N).

3.2.3. E-*2-(5,6-dimethyl-1*H-*benzotriazol-1-yl)-3-(4-bromophenyl)acrylonitrile (**5c**)*

This compound was obtained in 40% yield starting from **2b** and 4-bromobenzaldehyde (**4c**), after 24 h under reflux; m.p. 146–148 °C (acetone); ^{1}H NMR ($CDCl_3$): δ 7.88 (s, 1H, vinyl-H), 7.81–7.77 (m, 2H, H-4 + H-2′ + H-6′), 7.70–7.65 (m, 3H, H-7 + H-3′ + H-5′), 2.48 (s, 3H, CH_3), 2.44 (s, 3H, CH_3); MS: *m/z* 352/354 (M^+). Anal. $C_{17}H_{13}BrN_4$ (C, H, N).

3.2.4. E-*2-(5,6-dimethyl-1*H-*benzotriazol-1-yl)-3-(4-methylphenyl)acrylonitrile (**5d**)*

This compound was obtained in 55% yield starting from **2b** and 4-methylbenzaldehyde (**4d**), after 42 h under reflux; m.p. 136–138 °C (diethyl ether); ^{1}H NMR ($CDCl_3$): δ 7.86–7.81 (m, 4H, H-4 + vinyl-H + H-2′ + H-6′), 7.65 (s, 1H, H-7), 7.34 (d, 2H, J = 8.0 Hz, H-3′ + H-5′), 2.48 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.45 (s, 3H, CH_3); MS: *m/z* 288 (M^+). Anal. $C_{18}H_{16}N_4$ (C, H, N).

3.2.5. E-*2-(5,6-dimethyl-1*H-*benzotriazol-1-yl)-3-(4-methoxyphenyl)acrylonitrile (**5e**)*

This compound was obtained in 35% yield starting from **2b** and 4-methoxybenzaldehyde (**4e**), after 120 h under reflux; m.p. 195–197 °C (acetone); ^{1}H NMR ($CDCl_3$): δ 7.94–7.80 (m, 4H, H-4 + vinyl-H + H-2′ + H-6′), 7.65 (s, 1H, H-7), 7.04 (d, 2H, J = 8.6 Hz, H-3′ + H-5′), 3.91 (s, 3H, OCH_3), 2.47 (s, 3H, CH_3), 2.44 (s, 3H, CH_3); MS: *m/z* 304 (M^+). Anal. $C_{18}H_{16}N_4O$ (C, H, N).

3.2.6. E-*2-(5,6-dimethyl-1*H-*benzotriazol-1-yl)-3-(4-trifluoromethylphenyl)acrylonitrile (**5f**)*

This compound was obtained in 77% yield starting from **2b** and 4-trifluoromethylbenzaldehyde (**4f**), after 47 h under reflux; m.p. 153–154 °C (acetone); ^{1}H NMR ($CDCl_3$): δ 8.05–8.00 (m, 3H, vinyl-H + H-3′ + H-5′), 7.89 (s, 1H, H-4), 7.80 (d, 2H, J = 8.0 Hz, H-2′ + H-6′), 7.71 (s, 1H, H-7), 2.49 (s, 3H, CH_3), 2.46 (s, 3H, CH_3); MS: *m/z* 342 (M^+). Anal. $C_{18}H_{13}F_3N_4$ (C, H, N).

3.2.7. E-*2-(5,6-dimethyl-1*H-*benzotriazol-1-yl)-3-(3-nitrophenyl)acrylonitrile (**5g**)*

This compound was obtained in 59% yield starting from **2b** and 3-nitrobenzaldehyde (**4g**), after 24 h under reflux; m.p. 158–160 °C (acetone); ^{1}H NMR ($CDCl_3$): δ 8.69 (s, 1H, H-2′), 8.41–8.29 (m, 2H, H-4′ + H-5′), 8.06 (s, 1H, vinyl-H), 7.89 (s, 1H, H-4), 7.79 (d, 1H, J = 8.0 Hz, H-6′), 7.72 (s, 1H, H-7), 2.50 (s, 3H, CH_3), 2.46 (s, 3H, CH_3); MS: *m/z* 319 (M^+). Anal. $C_{17}H_{13}N_5O_2$ (C, H, N).

3.2.8. E-*2-(5,6-dimethy-1*H-*benzotriazol-1-yl)-3-(3-trifluoromethylphenyl)acrylonitrile (**5h**)*

This compound was obtained in 57% yield starting from **2b** and 3-trifluoromethylbenzaldehyde (**4h**), after 25 h under reflux; m.p. 138–139 °C (acetone); ^{1}H NMR ($CDCl_3$): δ 8.18 (d, 1H, J = 8.0 Hz, H-4′), 8.07 (s, 1H, H-2′), 8.00 (s, 1H, vinyl-H), 7.86 (s, 1H, H-4), 7.81–7.69 (m, 3H, H-7 + H-5′ + H-6′), 2.49 (s, 3H, CH_3), 2.45 (s, 3H, CH_3); MS: *m/z* 342 (M^+). Anal. $C_{18}H_{13}F_3N_4$ (C, H, N).

3.2.9. E-*2-(5-trifluoromethyl-1*H-*benzotriazol-1-yl)-3-(3-nitrophenyl)acrylonitrile (**5i**)*

This compound was obtained in 48% yield starting from **2c** and 3-nitrobenzaldehyde (**4g**), after reflux for 24 h; m.p. 168–170 °C (diethyl ether); ^{1}H NMR ($CDCl_3$): δ 8.73 (d, 1H, J = 1.0 Hz, H-4), 8.50 (d, 1H, J = 0.8 Hz, H-2′), 8.43 (dd, 1H, J = 8.0 and 1.0 Hz, H-6), 8.34 (dd, 1H, J = 8.0 and 0.8 Hz, H-7), 8.17 (s, 1H, vinyl-H), 8.14 (d, 1H, J = 8.8 Hz, H-4′), 7.92 (dd, 1H, J = 8.8 and 0.8 Hz, H-6′), 7.80 (t, 1H, J = 8.0 Hz, H-5′); MS: *m/z* 359 (M^+). Anal. $C_{16}H_8F_3N_5O_2$ (C, H, N).

3.2.10. E-*2-(6-trifluoromethyl-1*H-*benzotriazol-1-yl)-3-(3-nitrophenyl)acrylonitrile (**5j**)*

This compound was obtained in 67% yield from **2d** and 3-nitrobenzaldehyde (**4g**), after 16 h under reflux; m.p. 172–173 C (acetone/hexane); ^{1}H NMR ($CDCl_3$): δ 8.72 (d, 1H, J = 1.0 Hz, H-7), 8.46–8.29 (m, 5H, H-4 + H-5 + H-2′ + H-4′ + H-6′), 8.23 (s, 1H, vinyl-H), 7.81 (t, 1H, J = 8.0 Hz, H-5′); MS: *m/z* 359 (M^+). Anal. $C_{16}H_8F_3N_5O_2$ (C, H, Cl, N).

3.2.11. E-*2-(5-trifluoromethyl-1*H-*benzotriazol-1-yl)-3-(4-methoxyphenyl)acrylonitrile (**5k**)*

This compound was obtained in 25% yield from **3c** and 4-methoxybenzaldehyde (**4e**), after 40 h under reflux; m.p. 126–127 °C (diethyl ether); ^{1}H NMR ($CDCl_3$): δ 8.46 (s, 1H, H-4), 8.05–7.83 (m, 5H, H-6 + H-7 + H-2′ + H-6′ + vinyl-H), 7.06 (d, 2H, J = 8.8 Hz, H-3′ + H-5′), 3.93 (s, 1H, CH_3); MS: *m/z* 344 (M^+). Anal. $C_{17}H_{11}F_3N_4O$ (C, H, N).

3.2.12. E-*2-(5,6-dimethyl-1*H-*benzotriazol-1-yl)-3-(3,4-methylenedioxyphenyl)acrylonitrile (**5l**)*

This compound was obtained in 40% yield from **2b** and 3,4-(methylenedioxy)benzaldehyde (**4l**), after 96 h under reflux; m.p. 181–183 °C (acetone); ^{1}H NMR ($CDCl_3$): δ 7.85 (s, 1H, H-4), 7.77 (s, 1H, vinyl-H), 7.61 (dd, 1H, J = 8.2 and 1.6 Hz, H-6′), 7.35–7.30 (m, 2H, H-7 + H-2′), 6.94 (d, 1H, J = 8.2 Hz, H-5′), 6.11 (s, 2H, CH_2), 2.47 (s, 3H, CH_3), 2.44 (s, 3H, CH_3); MS: *m/z* 318 (M^+). Anal. $C_{18}H_{14}N_4O_2$ (C, H, N).

3.2.13. E-*2-(5-trifluoromethyl-1*H-*benzotriazol-1-yl)-3-(3,4-methylenedioxyphenyl)acrylonitrile (**5m**)*

This compound was obtained in 45% yield starting from **2c** and 3,4-(methylenedioxy)benzaldehyde (**4l**) heated for 24 h under reflux, then an extra portion of **4l** was added and the reflux continued for an additional 4 h; m.p. 165–166 °C (diethyl ether); ^{1}H NMR ($CDCl_3$): δ 8.47 (s, 1H, H-4), 8.03 (d, 1H, J = 8.8 Hz, H-6), 7.88–7.83 (m, 2H, H-7 + vinyl-H), 7.63 (d, 1H, J = 1.6 Hz, H-2′), 7.36 (dd, 1H, J = 8.4 and 1.6 Hz, H-6′), 6.96 (d, 1H, J = 8.0 Hz, H-5′), 6.13 (s, 2H, CH_2); MS: *m/z* 358 (M^+). Anal. $C_{17}H_9F_3N_4O_2$ (C, H, N).

3.2.14. E-*2-(2*H-*benzotriazol-2-yl)-3-(2-iodophenyl)acrylonitrile (**7a**)*

This compound was obtained in 51% yield starting from **3a** and 2-iodobenzaldehyde (**6a**), after 28 h under reflux; m.p. 148–149 °C (acetone/hexane); ^{1}H NMR ($CDCl_3$): δ 8.68 (s, 1H, vinyl-H), 8.07–7.90 (m, 4H, H-4 + H-7 + 2 phenyl-H), 7.57–7.44 (m, 3H, H-5 + H-6 + 1 phenyl-H), 7.21 (t, 1H, J = 7.8 Hz, H-5′); MS: *m/z* 372 (M^+). Anal. $C_{15}H_9IN_4$ (C, H, N).

3.2.15. E-*2-(2*H-*benzotriazol-2-yl)-3-(3-fluorophenyl)acrylonitrile (**7b**)*

This compound was obtained in 50% yield starting from **3a** (1.2 g, 8.0 mmol) and 3-fluorobenzaldehyde (**6b**) heated for 24 h under reflux, then an extra portion of **6b** was added and the reflux continued for an additional 4 h; m.p. 152–153 °C (acetone/hexane); ^{1}H NMR ($CDCl_3$): δ 8.48 (s, 1H, vinyl-H), 7.95–7.88 (m, 2H, H-4 + H-7), 7.78–7.68 (m, 2H, H-4′ + H-6′), 7.57–7.45 (m, 3H, H-5 + H-6 + H-2′), 7.26 (t, 1H, J = 8.2 Hz, H-5′); MS: *m/z* 264 (M^+). Anal. $C_{15}H_9FN_4$ (C, H, N).

3.2.16. E-*2-(5,6-dimethyl-2*H-*benzotriazol-2-yl)-3-(3,4-dimethoxyphenyl)acrylonitrile (**7c**)*

This compound was obtained in 52% yield from **3b** and 3,4-dimethoxybenzaldehyde (**6c**), after 48 h under reflux; m.p. 179–180 °C (acetone); ^{1}H NMR ($CDCl_3$): δ 8.37 (s, 1H, vinyl-H), 7.73 (d, 1H, J = 1.8 Hz, H-2′), 7.63 (s, 2H, H-4 + H-7), 7.47 (dd, 1H, J = 8.0 and 1.8 Hz, H-6′), 6.97 (d, 1H,

J = 8.0 Hz, H-5′), 3.99 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 2.43 (s, 6H, 2 CH_3); MS: *m/z* 334 (M^+). Anal. $C_{19}H_{18}N_4O_2$ (C, H, N).

3.2.17. E-*2-(5-trifluoromethyl-2*H-*benzotriazol-2-yl)-3-(4-methoxyphenyl)acrylonitrile (**7d**)*

This compound was obtained in 50% yield starting from **3c** and 4-methoxybenzaldehyde (**4e**), after 24 h under reflux; m.p. 133–134 °C (acetone); ^{1}H NMR ($CDCl_3$): δ 8.52 (s, 1H, H-4), 8.27 (s, 1H, vinyl-H), 8.0–7.98 (m, 3H, H-7 + H-2′ + H-6′), 7.64 (d, 1H, J = 8.6 Hz, H-6), 7.05 (d, 2H, J = 8.4 Hz, H-3′ + H-5′), 3.92 (s, 3H, OCH_3); MS: *m/z* 344 (M^+). Anal. $C_{17}H_{11}F_3N_4O$ (C, H, N).

3.2.18. E-*2-(5-trifluoromethyl-2*H-*benzotriazol-2-yl)-3-(3-nitrophenyl)acrylonitrile (**7e**)*

This compound was obtained in 48% yield starting from **3c** and 3-nitrobenzaldehyde (**4g**), after 18 h under reflux; m.p. 166–167 °C (acetone); ^{1}H NMR ($CDCl_3$): δ 8.74 (s, 1H, H-4), 8.66 (s, 1H, vinyl-H), 8.44–8.29 (m, 2H, H-7 + H-4′), 8.10 (s, 1H, H-2′), 8.08 (d, 1H, J = 8.8 Hz, H-6′), 7.79 (t, 1H, J = 8.4 Hz, H-5′), 7.68 (d, 1H, J = 8.4 Hz, H-6); MS: *m/z* 359 (M^+). Anal. $C_{16}H_8F_3N_5O_2$ (C, H, N).

3.2.19. E-*2-(5-trifluoromethyl-2*H-*benzotriazol-2-yl)-3-(3,4-methylenedioxyphenyl)acrylonitrile (**7f**)*

This compound was obtained in 40% yield starting from **3c** and 3,4-(methylenedioxy)benzaldehyde (**4l**), after 28 h under reflux; m.p. 160–161 °C (acetone); ^{1}H NMR ($CDCl_3$): δ 8.47 (s, 1H, H-4), 8.27 (s, 1H, vinyl-H), 8.05 (d, 1H, J = 8.8 Hz, H-7), 7.69–7.62 (m, 2H, H-6 + H-2′), 7.44 (d, 1H, J = 8.0 Hz, H-6′), 6.96 (d, 1H, J = 8.0 Hz, H-5′), 6.12 (s, 2H, CH_2); MS: *m/z* 358 (M^+). Anal. $C_{17}H_9F_3N_4O_2$ (C, H, Cl, N).

3.3. Microbiological assays

3.3.1. Compounds

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

3.3.2. 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 penicillin G and 100 μg/ml 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.

3.3.3. Antiproliferative assays

Exponentially growing 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 [15].

3.3.4. Anti-mycobacterial assays

M. tuberculosis ATCC 27294 (wt), *M. tuberculosis* ATCC 35822 (INH®), *M. tuberculosis* ATCC 35820 (Str.®) and *M. tuberculosis* ATCC 35828 (Pyr.®) were from American Type Culture Collection (ATCC). Minimum inhibitory concentrations (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 [15].

4. Results and discussion

The new 3-aryl-2-(1*H*-benzotriazol-1-yl)acrylonitriles **5a–m**, and 3-aryl-2-(2*H*-benzotriazol-2-yl)acrylonitriles **7a–f**, reported in Schemes 2 and 3 and Tables 2 and 3, were evaluated for cytotoxicity against MT-4 cells and for antitubercular activity against some strains of *M. tuberculosis*.

The cytotoxicity of **5a–m** and **7a–f** against MT-4 cells is reported in Table 4. The most active derivatives (CC_{50} < 3.0 μM) (**5a,g,i,j,l** and **7b**) were then evaluated against a panel of human cell lines derived from hematologi-

Table 4
Cytotoxicity of compounds (**5a–m** and **7a–f**)

Compound	CC_{50} MT-4 [a]	Compound	CC_{50} MT-4 [a]	Compound	CC_{50} MT-4 [a]
5a	1.9	**5h**	13	**7b**	2.8
5b	24	**5i**	2.0	**7c**	>100
5c	7	**5j**	0.7	**7d**	65
5d	6	**5k**	69	**7e**	53
5e	17	**5l**	1.3	**7f**	>100
5f	13	**5m**	≥100		
5g	0.5	**7a**	10		

[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 three separate experiments. Variation among duplicate samples was less than 15%.

Table 5
Activity against leukemia/lymphoma-derived cell lines of compounds (**5a,g,i,j,l** and **7b**)

Compound	IC_{50} (μM) [a]		
	CCRF-CEM [b]	WIL-2NS [c]	CCRF-SB [d]
5a	3.0 ± 0.2	6.8 ± 0.3	3.6 ± 0.2
5g	0.6 ± 0.001	1.4 ± 0.05	1.1 ± 0.1
5i	1.5	2.0 ± 0.05	1.7 ± 0.3
5j	0.5 ± 0.002	1.0 ± 0.05	0.7 ± 0.2
5l	4.4 ± 0.6	5.0 ± 0.4	3.2 ± 0.4
7b	4.4 ± 0.5	6.2 ± 0.3	4.8 ± 0.4
6MP	1.0	3.1	1.1
Etoposide	0.09	0.1	0.2

[a] Compound concentration required to reduce cell proliferation by 50%, as determined by the MTT method, under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication. Data represent mean ± S.D. for three independent determinations.
[b] $CD4^+$ human acute T-lymphoblastic leukemia.
[c] Human splenic B-lymphoblastoid cells.
[d] Human acute B-lymphoblastic leukemia.

Table 6
Activity against solid human tumor-derived cell lines of compounds (**5a,g,i,j,l** and **7b**)

Compound	IC_{50} (μM) [a]				
	SK-MEL-28 [b]	MCF7 [c]	SKMES-1 [d]	HepG2 [e]	DU145 [f]
5a	6.4	5.6	5.3	7.2	4.1
5g	2.0	2.5	2.6	3.4	1.9
5i	3.3	3.7	6.5 ± 0.2	3.3 ± 0.1	3.2
5j	2.5	5.3	6.0 ± 0.6	4.2	2.7
5l	13.7	5.6	15.6	18.3	11.2
7b	6.4	24.5	6.5	11.1	7.1
6MP	15	3.2	58.0	8.0	2.0
Etoposide	1.2	1.0	0.3	0.7	0.4

[a] Compound concentration required to reduce cell proliferation by 50%, as determined by the MTT method, under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication. Data represent mean values for three independent determinations. 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.

cal (CCRF-CEM, WIL-2NS and CCRF-SB) and solid (SK-MEL28, MCF7, SKMES-1, HepG2 and DU145) tumors. The results, reported in Tables 5 and 6, respectively, show that the compounds (**5g,i,j**) were the most active ones against hematological and solid tumors. In particular compound (**5g**) resulted more potent than 6-mercaptopurine (6-MP) on all cell lines, and 2–14-fold less potent than etoposide. These data, in comparison to the benzo-unsubstituted derivatives described in previous series [3], show that the introduction of methyl groups in both 5 and 6 positions in the benzo-moiety together with a nitro group in 3′ position in the phenyl-moiety of 3-phenyl-2-(1*H*-benzotriazol-1-yl)acrylonitriles increases the antiproliferative activity. This trend was not confirmed when position 5 or 6 and 3′ were substituted, respectively, with a trifluoromethyl and nitro groups.

All compounds were submitted to preliminary antitubercular screening against *M. tuberculosis* ATCC 27294. The derivatives which exhibited MIC_{50} < 100 μM were tested against resistant strains of *Mycobacterium* (*M. tuberculosis* ATCC 35822 INH®, *M. tuberculosis* ATCC 35820 Str.® and *M. tuberculosis* ATCC 35828 Pyr.®), the results are showed in Table 7.

The derivatives **5i** and **5j** showed moderate activity against the strains tested and in any case they were cytotoxic for MT-4 cells at concentrations lower than those inhibiting mycobacteria. **7e** only was somewhat active against mycobacterial strains but at concentrations comparable to those eliciting cytotoxicity. The latter observation seems to confirm our hypothesis about a better selectivity of the 3-aryl-2-(2*H*-benzotriazol-2-yl)acrylonitriles in comparison of the 3-aryl-2-(1*H*-benzotriazol-1-yl)acrylonitriles against resistant strains of *Mycobacterium*. The analytical data for all compounds is given in Table 8.

Table 7
Anti-mycobacterial activity of compounds (**5a,i,j** and **7e**)

Compound	MIC_{50} [a]/MIC_{90} [b]			
	M. tuberculosis ATCC 27294 (wt)	*M. tuberculosis* ATCC 35820 (Str.®)	*M. tuberculosis* ATCC 35822 (INH®)	*M. tuberculosis* ATCC 35828 (Pyr.®)
5a	65.5/>100	ND	ND	ND
5i	75.9/>100	>100	89.4/>100	84.9/>100
5j	39.6/84.0	41.3/85.0	35.7/81.4	19.2/82.9
7e	51.5/>100	38.6/>100	36.1/>100	53.5/>100
Ciprofloxacin	0.96/2.9	1.4/3.3	1.5/3.7	0.6/3.0
Ofloxacin	0.96/2.7	1.4/3.1	1.5/3.3	0.9/3.0
Isoniazid	0.06/0.09	0.05/0.09	>100	0.04/0.09
Ethambutol	1.4/10.3	4.7/21.0	4.9/24.5	1.8/19.4
Streptomycin	0.02/0.09	>100	0.05/0.09	0.012/0.1
Rifampin	0.12/0.7	0.7/1.1	0.2/0.8	0.17/0.8
Pyrazinamide	>100	>100	>100	>100

ND: Not determined.
[a] Minimum inhibitory concentration (μM) required to reduce the number of viable mycobacteria by 50%, as determined by the MTT method.
[b] 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 8
Analytical data

Compound	Empirical formula	Calculated (%)			Found (%)		
		C	H	N	C	H	N
2b	$C_{10}H_{10}N_4$	64.50	5.41	30.09	64.82	5.23	29.87
3b	$C_{10}H_{10}N_4$	64.50	5.41	30.09	64.33	5.49	30.36
2c	$C_9H_5F_3N_4$	47.80	2.23	24.77	48.15	2.01	24.40
2d	$C_9H_5F_3N_4$	47.80	2.23	24.77	47.48	2.11	24.96
3c	$C_9H_5F_3N_4$	47.80	2.23	24.77	47.96	2.56	25.11
5a	$C_{17}H_{14}N_4$	74.43	5.22	20.42	74.09	5.51	20.14
5b	$C_{17}H_{13}ClN_4$	66.13	4.24	18.14	65.84	4.01	18.41
5c	$C_{17}H_{13}BrN_4$	57.81	3.71	15.86	58.20	3.49	15.46
5d	$C_{18}H_{16}N_4$	74.98	5.59	19.45	74.62	5.90	19.82
5e	$C_{18}H_{16}N_4O$	71.04	5.30	18.41	71.43	5.06	18.09
5f	$C_{18}H_{13}F_3N_4$	63.16	3.83	16.37	63.00	4.01	16.76
5g	$C_{17}H_{13}N_5O_2$	63.94	4.10	21.93	64.32	4.21	21.80
5h	$C_{18}H_{13}F_3N_4$	63.16	3.83	16.37	63.50	3.62	16.04
5i	$C_{16}H_8F_3N_5O_2$	53.49	2.24	19.49	53.80	2.51	19.73
5j	$C_{16}H_8F_3N_5O_2$	53.49	2.24	19.49	53.86	2.12	19.88
5k	$C_{17}H_{11}F_3N_4O$	59.31	3.22	16.27	59.03	3.00	16.58
5l	$C_{18}H_{14}N_4O_2$	67.92	4.43	17.68	68.28	4.49	17.41
5m	$C_{17}H_9F_3N_4O_2$	56.99	2.53	15.64	56.74	2.80	15.40
7a	$C_{15}H_9IN_4$	48.41	2.44	15.05	48.12	2.40	15.35
7b	$C_{15}H_9FN_4$	68.18	3.43	21.20	68.44	3.56	20.89
7c	$C_{19}H_{18}N_4O_2$	68.25	5.43	16.76	68.64	5.33	17.02
7d	$C_{17}H_{11}F_3N_4O$	59.31	3.22	16.22	59.07	3.29	16.53
7e	$C_{16}H_8F_3N_5O_2$	53.49	2.24	19.49	53.40	2.39	19.56
7f	$C_{17}H_9F_3N_4O_2$	56.99	2.53	15.64	56.68	2.50	15.43

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