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Short communication

Synthesis and antitubercular activity of 3-aryl substituted-2-(1*H*(2*H*) benzotriazol-1(2)-yl)acrylonitriles[#]

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Abstract – A series of 22 3-aryl substituted-2-(1*H*(2*H*)-benzotriazol-1(2)-yl)acrylonitriles was synthesized for a preliminary in vitro evaluation of antitubercular activity according to an international program with the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF). This work reports the synthetic approach and analytical and spectroscopic characterization (UV, IR, ^{1}H- and ^{13}C-NMR) of all compounds synthesized. Several compounds showed an interesting activity in the preliminary screening with a percent growth inhibition of the virulent *Mycobacterium tuberculosis* between 40 and 99% at the concentration of 12.5 µg/mL. The most effective derivatives *E*-**5a** and *E*-**5e** were also tested against *M. avium* in vitro. © 2000 Éditions scientifiques et médicales Elsevier SAS

antimycobacterial activity / 3-aryl substituted-2-(1*H*(2*H*)-benzotriazol-1(2)-yl)acrylonitriles / Knoevenagel condensation

1. Introduction

Tuberculosis today still represents one of the major problems for public health world-wide. After a long period in which it seemed to be declining, in the last two decades an unexpected return has been recorded for this disease [1, 2]. This resurgence affected both in developing countries, where infection is endemic, and industrialized countries. In particular, it is generally well accepted that factors which determined the return of tuberculosis in industrialized countries are: the migrant flow from low income countries, the diffusion of HIV infection and the development of multidrug-resistance of *Mycobacterium* strains. The World Health Organization has estimated that every year about eight million new cases of tuberculosis occur and up to three million individuals die due to this disease [3]. Isoniazid, pyrazinamide, ethambutol, rifampicin and streptomycin, generally used in combination among them, are still today the drugs of choice in the therapy of tuberculosis [4]. However the protracted use in time of these molecules represents the main cause of outbreak of new resistant strains. Therefore, there is an urgent need for the development of new drugs having a mechanism different from those mentioned above. Unfortunately, at present, with the exception of rifampicin, little is known about the mechanism of action and the structure–activity relationship of these drugs and that represents an important obstruction to a rational approach of the research in this field. In spite of these difficulties, the numerous reports in the literature of the last years on this matter give in evidence the renewed interest of researchers for antitubercular agents. Among a large amount of molecules tested for this purpose the heterocycles benzofurane [5], benzothiazole [6, 7], benzoisothiazole [8] and benzimidazole [9–11] have to be cited for their interesting and promising results. Thus, with this in mind we have taken into account the bioisosteric replacement of those heterocycles with a benzotriazole ring, as a part of our continuous interest for both chemical [12–15] and biological properties [16–19] of this heterocycle. For this project, developed in agreement with an international program with the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF), we designed a series of 3-aryl

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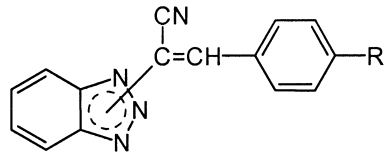

Figure 1. 3-aryl derivatives of 2-(1*H*(2*H*)-benzotriazol-1(2)-yl)acrylonitriles.

derivatives of 2-(1*H*(2*H*)-benzotriazol-1(2)-yl)acrylonitriles of *figure 1* which were tested at the Southern Research Institute, GWL Hansen's Disease Center, Colorado (USA), against *Mycobacterium tuberculosis* in order to discover a new lead for the development of a new class of antitubercular agents.

In this paper we report the preparation of compounds **5a–i** and **6a–i** (*figure 2*) which represent the first part of this project in which we considered preliminarily the characterization of the best substituents connected with the aryl moiety. For this purpose the nature of R was selected within groups with various electron-accepting or donor properties and lipophilic–hydrophilic balance. In particular the electronegative substituents F, Cl, Br and the most electron-withdrawing NO_2 and CF_3 groups were compared either with unsubstituted terms or with those bearing electron-releasing groups such as CH_3 and NH_2. Finally, a COOH group was also introduced which possesses both electron-withdrawing and hydrophilic properties. On the whole the structural features of these compounds allowed us a preliminary evaluation of the structure–activity relationship.

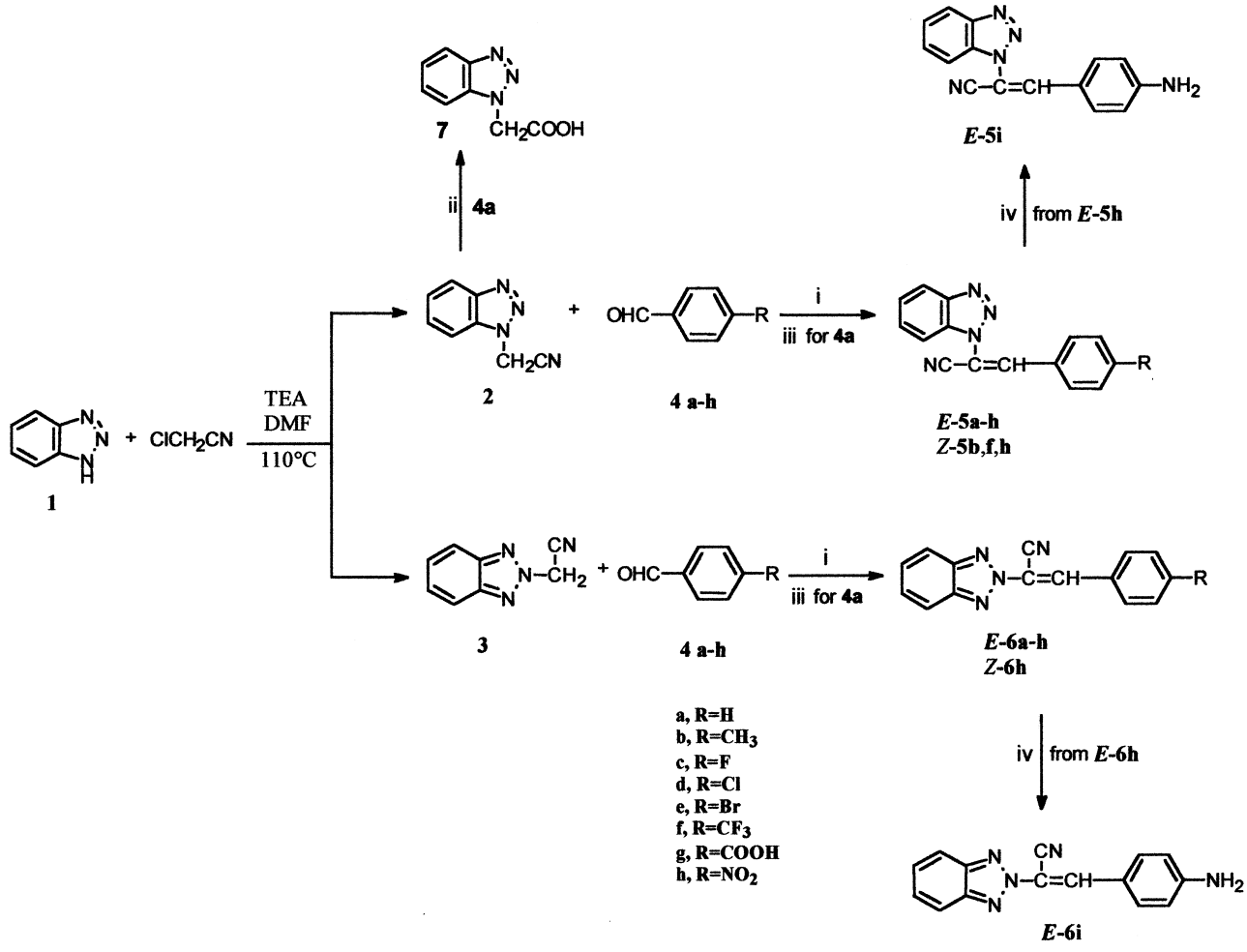

Figure 2. (i) TEA/toluene/reflux, (ii) Na/ethanol/reflux, (iii) piperidine/toluene/reflux, (iv) Fe/H^+.

2. Chemistry

The desired compounds **5a–h** and **6a–h** were synthesized according to the sequence of reactions depicted in *figure 2*, by Knoevenagel condensation of the appropriate 2-(1*H*-benzotriazol-1-yl)- **2** or 2-(2*H*-benzotriazol-2-yl)acetonitrile **3** with the suitable *para*-substituted benzaldehyde **4a–h** in refluxing toluene, using triethylamine (TEA) as basic catalyst.

This approach represents a straightforward method for preparation of these compounds, taking advantage of the acidic character of the methylene group of the intermediate **2** or **3**, activated by both cyano group and benzotriazolyl moiety. Formation of these acrylonitriles is very critical where different basic catalysts are used. The reaction conditions have been optimized by us in the case of preparation of compounds **5a** and **6a** and the procedure was then extended to all compounds. Thus, we observed that when sodium in ethanol was used in the reaction of **2** with **4a** the only known 2-(1*H*-benzotriazol-1-yl)acetic acid **7** [20] was isolated, while when the same condensation was carried out in toluene in the presence of piperidine, afforded compounds **5a** and **6a** in lower yields. Although from the Knoevenagel condensation two geometric isomers may be obtained, we observed that in most cases the *E*-isomer was the only one formed, while in a few cases a mixture of *E/Z*-isomers was obtained. The amines *E*-**5i** and *E*-**6i** were prepared by reduction of the parent nitro-derivatives *E*-**5h** and *E*-**6h**, respectively. The intermediates **2** and **3** were known and have been prepared by condensation of the chloroacetonitrile with 1*H*-benzotriazole **1** in DMF at reflux in the presence of triethylamine, modifying the method previously described by Danan et al. [21].

Separation of the isomeric mixtures was performed by column chromatography and the structures to the single isomers were assigned on the basis of their UV, ^{1}H- and ^{13}C-NMR spectra. In particular, by using the 'gate decoupling' technique a ^{13}C–H coupling was evidenced for vinylic-H with the nitrilic-C and, according to the data of the literature [22, 23], the *E*-isomers showed high values of $^{3}J_{C-H}$ constants (12–13 Hz) while those of the *Z*-isomers were much lower (0–6 Hz). Moreover, we observed that the vinylic-H in 1*H*-benzotriazol-1-yl-derivatives *E*-**5a–i** resonates as a singlet at higher field (δ 8.38–7.65) in comparison with that of 2*H*-benzotriazol-2-yl derivatives *E*-**6a–i** (δ 8.83–8.38). Furthermore, we also observed, within the *E/Z* isomeric pairs of two series of compounds, that the vinylic-H of the *E*-isomers exhibited a down-field shift (δ 8.81–7.65) compared to the *Z*-isomers (δ 7.60–6.03). In addition, the UV spectra in ethanol of compounds *E*-**6a–i** showed a single absorption maximum in the range 289–422 nm while isomers *E*-**5a–i** exhibited two absorption maxima ranging between 388–317 and 293–254 nm, in accordance with previous reports for similar cases [24]. An analogous bathochromic effect (30–60 nm) was also observed in two series of derivatives for the *E*-isomers compared to the *Z*-isomers.

3. Pharmacology

All described compounds were tested in vitro for their antitubercular activity at the GWL Hansen's Disease Center (Colorado State University) within the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF) screening program for the discovery of novel drugs for treatment of tuberculosis. The purpose of the screening program is to provide a resource whereby new experimental compounds can be tested for their capacity to inhibit the growth of virulent *M. tuberculosis*.

4. Results

Results of the in vitro evaluation of antitubercular activity are reported in *table I* and they show an interesting activity for several compounds. Compounds *E*-**5a**, *E*-**5b**, *E*-**5d**, *E*-**5e**, *E*-**5f**, *E*-**5h**, *Z*-**5h**, *E*-**6a** and *E*-**6h** exhibited a growth inhibition against *M. tuberculosis* at a concentration of 12.5 μg/mL in the range 40–99%. These data were compared with those of rifampicin, as reference drug, that showed an inhibition activity of 98% at a concentration of 0.25 μg/mL. Compounds *E*-**5a** and *E*-**5e**, which exhibited a growth inhibition greater than 90% were selected for confirmatory and advanced screening in order to determine the actual MIC and their activity against the opportunistic pathogen *M. avium* in comparison with clarithromycin. Results of the latter tests are reported in *table II*.

These data show, for the phenyl derivative *E*-**5a**, an actual MIC = 6.25 μg/mL (IC_{50} = 2 μg/mL), while the 4-bromophenyl derivative *E*-**5e** exhibited an MIC = 12.5 μg/mL. Results of the primary screening against *M. avium* demonstrated a low activity for compound *E*-**5a** (growth inhibition of 30% at a concentration of 12.5 μg/mL), while compound *E*-**5e** resulted the most interesting having an inhibitory activity of 93% at the same concentration.

5. Discussion

In spite of the restricted number of compounds tested in this first study, some preliminary considerations of

Table I. In vitro evaluation of antitubercular activity as % growth inhibition at 12.5 μg/mL concentration (rifampicin MIC = 0.25 μg/mL) versus *Mycobacterium tuberculosis*.

Compound	MIC (μg/mL)	% inhibition	Compound	MIC (μg/mL)	% inhibition
E-**5a**	< 12.5	98	*E*-**6a**	> 12.5	76
E-**5b**	> 12.5	55	*E*-**6b**	> 12.5	31
E-**5c**	> 12.5	5	*E*-**6c**	> 12.5	0
E-**5d**	> 12.5	86	*E*-**6d**	> 12.5	12
E-**5e**	< 12.5	99	*E*-**6e**	> 12.5	6
E-**5f**	> 12.5	82	*E*-**6f**	> 12.5	17
E-**5g**	> 12.5	0	*E*-**6g**	> 12.5	6
E-**5h**	> 12.5	43	*E*-**6h**	> 12.5	67
Z-**5h**	> 12.5	49	*Z*-**6h**	> 12.5	31
E-**5i**	> 12.5	33	*E*-**6i**	> 12.5	28

structure–activity relationships can be put forward. From analysis of the data reported in *table I*, it can be generally deduced that 1-substituted benzotriazole derivatives **5** resulted more active than the 2-benzotriazolyl isomers **6**, with the only exception for the compound *E*-**6h**. At the present stage of research no deduction can be advanced about the influence of the geometric isomerism (*E/Z*) on the biological activity owing to the exiguousness of the *Z*-isomers obtained and tested. The effect of substituents present in the phenyl moiety were observed within each series of all substituted derivatives which resulted less active than the unsubstituted terms (*E*-**5a** and *E*-**6a**) with the exception of *E*-**5e**, which in turn exhibited the highest antimycobacterial activity in vitro and also demonstrated a good activity against *M. avium*. Furthermore, the data of biological activity seem to show that a balance between both lipophilic and electronegative effects of the substituent in the phenyl moiety is necessary to determine an appreciable antimycobacterial activity. That appears evident from results of compounds *E*-**5d**, *E*-**5e** and *E*-**5f** containing a Cl, Br or CF_3 group respectively. In fact when R is represented by both a hydrophilic electron-withdrawing group (COOH, NO_2) or an electron-releasing group with both lipophilic (CH_3) and hydrophilic (NH_2) properties, the activity resulted lower than that of the unsubstituted terms *E*-**5a** and *E*-**6a**. In the light of the above results we can conclude that the benzotriazole appears to be a useful substrate for antitubercular agents.

Table II. Actual MIC against *Mycobacterium tuberculosis* (rifampicin) and % growth inhibition against *M. avium* (clarithromycin) of compounds *E*-**5a** and *E*-**5e**.

Compound	Actual MIC against *M. tuberculosis* (μg/mL)	% inhibition against *M. avium* at 12.5 μg/mL
E-**5a**	6.25	30
E-**5e**	12.5	93
Rifampicin	0.125	–
Clarithromycin	–	98*

* Determined at 2 μg/mL.

6. Experimental protocols

6.1. Chemistry

Melting points were determined in open capillaries in a Digital Electrothermal IA9100 melting point apparatus and are uncorrected. IR spectra were recorded as nujol mulls with a Perkin-Elmer 781 spectrophotometer. UV spectra are qualitative and were recorded in nm for solutions in ethanol with a Perkin-Elmer Lambda 5 spectrophotometer. Nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra were obtained with a Varian XL-200 instrument (200 MHz for ^{1}H- and 50 MHz for ^{13}C-), using TMS as internal standard. 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), t (triplet), q (quadruplet), m (multiplet), and br s (broad singlet). MS spectra were performed on a combined HP 5790-HP 5970 GC/MS apparatus. Silica gel 60 (Merck 70–230 and 230–400 mesh silica gel) was used for column chromatography. The progress of the reactions and the purity of the final compounds was monitored by TLC using Merck F-254 commercial plates. Elemental analyses were performed by the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, Università di Padova (Padua). The analytical results for C, H, N, and halogen, when present, were within ± 0.4% of the theoretical values.

6.1.1. 2-(1H-Benzotriazol-1-yl)acetonitrile **2** *and 2-(2H-benzotriazol-2-yl)acetonitrile* **3**

To a stirred solution of benzotriazole (Aldrich) (10.0 g, 84 mmol) and triethylamine (9.46 g, 93 mmol) in DMF (30 mL), chloroacetonitrile (9.54 g, 126 mmol) was slowly added dropwise. After the addition was complete, the reaction mixture was stirred at 110 °C for 12 h and then allowed to reach room temperature. The resulting precipitate of triethylamine hydrochloride was filtered off and the filtrate evaporated to dryness under reduced pressure. The resulting residue was taken up with diethyl ether and washed twice with water. The organic layer was then dried (Na_2SO_4), evaporated and the solid residue obtained flash chromatographed on silica gel (eluent: diethyl ether–light petroleum 8:2) affording in sequence: 2-(*2H*-benzotriazol-2-yl)acetonitrile **3** (2.64 g, 20%), m.p. 78–79 °C (literature [21]: m.p. 78 °C), a 1:1 mixture of isomers **2** and **3** (0.33 g, 2.5%) and then 2-(*1H*-benzotriazol-1-yl)acetonitrile **2** (9.54 g, 71.9%), m.p. 86–87 °C (literature [21]: m.p. 87 °C).

6.1.2. General procedure for preparation of 2-(1H-benzotriazol-1-yl)-3-arylacrylonitriles **5a–h** *and 2-(2H-benzotriazol-2-yl)-3-arylacrylonitriles* **6a–h**

A mixture of **2** or **3** (6.3–12.6 mmol) and triethylamine (18.9–37.8 mmol) in toluene (25–30 mL) was stirred at room temperature for 20 min. Then a solution of the appropriate arylaldheyde **4a–h** (8–16 mmol) in toluene (10 mL) was slowly added and the reaction mixture was heated under reflux for the time indicated below. After removal of the solvent, the solid crude residue was purified according to the indications described below.

*6.1.2.1. E-2-(1H-Benzotriazol-1-yl)-3-phenylacrylonitrile E-***5a**

Method A: the title compound was obtained in 60% yield (0.93 g) after purification by chromatography (eluent: mixtures of diethyl ether–light petroleum from 1:1 to 7:3 ratio), starting from **2** (1 g, 6.3 mmol) and benzaldehyde **4a** (0.85 g, 8 mmol) after 8 h under reflux. M.p. 112–114 °C; IR (cm^{-1}): 2 260 (CN), 1 640, 1 600, 1 570; UV (nm): λ_{max} 317, 285, 222 sh, 208; ^{1}H-NMR ($CDCl_3$): δ 8.16 (d, 1H, *J* = 8.4, H-4), 7.96 (s, 1H, vinylic-H), 8.00–7.91 (m, 3H, H-7 + H-2′ + H-6′), 7.65 (dd, 1H, *J* = 7.4 and 1.2, H-6), 7.60–7.45 (m, 3H, H-3′ + H-4′ + H-5′), 7.50 (dd, 1H, *J* = 7.4 and 1.2, H-5); ^{13}C-NMR ($CDCl_3$): δ 146.31 (s), 140.51 (d), 131.94 (d), 131.65 (s), 130.44 (s), 129.61 (d, 2 CH), 129.32 (d, 2 CH), 129.11 (d), 125.08 (d), 120.64 (d), 113.55 (s, CN), 110.21 (d), 106.54 (s); MS *m/z* 246 (M^+). Anal. $C_{15}H_{10}N_4$ (C, H, N).

Method B: the title compound was also obtained in 25% yield (0.39 g) starting from **2** (1 g, 6.3 mmol), **4a** (0.71 g, 8.0 mmol) and piperidine (0.68 g, 18 mmol) in toluene (30 mL) after reflux for 8 h and purification by column chromatography on silica gel (eluent: diethyl ether–light petroleum 8:2) of the residue obtained from evaporation of the solvent.

*6.1.2.2. E-2-(2H-Benzotriazol-2-yl)-3-phenylacrylonitrile E-***6a**

Method A: starting from **3** (1 g, 6.3 mmol) and **4a** (1.29 g, 12.2 mmol) after 15 h under reflux and crystallization from acetone compound *E*-**6a** was obtained in 84% yield (1.30 g); m.p. 152–153 °C; IR (cm^{-1}): 2 230 (CN), 1 625, 1 590, 1 555; UV (nm): λ_{max} 339, 276, 264, 222; ^{1}H-NMR ($CDCl_3$): δ 8.53 (s, 1H, vinylic-H), 8.02–7.90 (m, 4H, H-4 + H-7 + H-2′ + H-6′), 7.56–7.45 (m, 5H, H-5 + H-6 + H-3′ + H-4′ + H-5′); ^{13}C-NMR ($CDCl_3$): δ 144.98 (s), 137.11 (d), 131.89 (d), 130.22 (s), 129.91 (d, 2 CH), 129.28 (d, 2 CH), 128.15 (d, 2 CH), 127.86 (s), 118.23 (d, 2 CH), 112.93 (s, CN), 112.17 (s); MS *m/z* 246 (M^+). Anal. $C_{15}H_{10}N_4$ (C, H, N).

Method B: the title compound was also obtained in 39% yield (0.60 g) starting from **3** (1 g, 6.3 mmol), **4a** (0.71 g, 8.0 mmol) and piperidine (0.68 g, 18 mmol) in toluene (30 mL) after reflux for 15 h and purification by column chromatography on silica gel (eluent: diethyl ether–light petroleum 7:3) of the residue obtained from evaporation of the solvent.

*6.1.2.3. (E/Z)-2-(1H-Benzotriazol-1-yl)-3-(4-methylphenyl) acrylonitrile E-***5b** *and Z-***5b**

Starting from **2** (2 g, 12.6 mmol) and 4-methylbenzaldheyde **4b** (1.48 g, 25.2 mmol) after 20 h under reflux, by trituration of the crude reaction residue with diethyl ether, compound *E*-**5b** was obtained in 19.6% yield (0.64 g,); m.p. 98–100 °C; IR (cm^{-1}): 2 220 (CN), 1 600, 1 580; UV (nm): λ_{max} 324, 292, 231, 205; ^{1}H-NMR ($CDCl_3$): δ 8.14 (d, 1H, *J* = 8.4, H-4), 7.89 (s, 1H, vinylic-H), 7.91 (d, 1H, *J* = 8.4, H-7), 7.84 (d, 2H, *J* = 8.2, H-2′ + H-6′), 7.63 (dd, 1H, *J* = 7.4 and 1.2, H-6), 7.49 (dd, 1H, *J* = 7.4 and 1.2, H-5), 7.34 (d, 2H, *J* = 8.2, H-3′ + H-5′), 2.45 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): δ 145.32 (s), 142.25 (s), 141.20 (d), 131.46 (s), 129.71 (d, 2 CH), 129.51 (d, 2 CH), 129.14 (d), 127.73 (s), 125.18 (d), 119.87 (d), 114.10 (s, $^3J_{C-H}$ = 12.3, CN), 110.85 (d), 105.03 (s), 21.09 (t); MS *m/z* 260 (M^+). Anal. $C_{16}H_{12}N_4$ (C, H, N).

Chromatography of the residue obtained from evaporation of the ethereal mother liquors (eluent: diethyl ether–light petroleum 7:3) afforded a further amount of *E*-**5b** (0.4 g, 12.2%) accompanied with compound *Z*-**5b** (0.07 g, 2.1%) as white needles with m.p. 152–153 °C; IR (cm^{-1}): 2 225 (CN), 1 630, 1 610; UV (nm): λ_{max} 302 infl,

293, 264 infl, 230, 207; ^{1}H-NMR ($CDCl_3$): δ 8.17 (d, 1H, *J* = 7.4, H-4), 7.60 (s, 1H, vinylic-H), 7.55–7.46 (m, 2H, H-5 + H-6), 7.36 (d, 1H, *J* = 7.4, H-7), 7.00 (d, 2H, *J* = 7.4, H-2′ + H-6′), 6.75 (d, 2H, *J* = 7.4, H-3′ + H-5′), 2.27 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): δ 145.66 (s), 145.03 (s), 142.62 (d), 131.46 (s), 129.95 (d, 2 CH), 129.61 (d, 2 CH), 129.56 (d), 127.77 (s), 125.35 (d), 120.07 (d), 115.70 (s, $^3J_{C-H}$ = 6.0, CN), 110.16 (d), 103.88 (s), 20.94 (t); MS *m/z* 260 (M^+). Anal. $C_{16}H_{12}N_4$ (C, H, N).

*6.1.2.4. E-2-(2H-Benzotriazol-2-yl)-3-(4-methylphenyl)acrylonitrile E-***6b**

This compound was prepared in 89% yield (0.73 g) starting from **3** (0.5 g, 3.1 mmol) and **4b** (2.27 g, 18.9 mmol) after refluxing for 8 h and cooling at room temperature; m.p. 182–184 °C; IR (cm^{-1}): 2 220 (CN), 1 580, 1 550; UV (nm): λ_{max} 346, 279, 235, 225, 203; ^{1}H-NMR ($CDCl_3$): δ 8.47 (s, 1H, vinylic-H), 8.00–7.80 (m, 4H, H-4 + H-7 + H-2′ + H-6′), 7.47–7.42 (m, 2H, H-5 + H-6), 7.32 (d, 2H, *J* = 8.2, H-3′ + H-5′), 2.44 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6): δ 144.56 (s), 142.82 (s), 138.00 (d), 130.01 (d, 2 CH), 129.95 (d, 2 CH), 128.40 (d, 2 CH), 127.62 (s), 118.03 (d, 2 CH), 117.91 (s), 113.13 (s, CN), 110.87 (s), 20.93 (t); MS *m/z* 260 (M^+). Anal. $C_{16}H_{12}N_4$ (C, H, N).

*6.1.2.5. E-2-(1H-Benzotriazol-1-yl)-3-(4-fluorophenyl)acrylonitrile E-***5c**

This compound was obtained in 67.9% yield (2.26 g) starting from **2** (2 g, 12.6 mmol) and 4-fluorobenzaldheyde **4c** (1.17 g, 9.45 mmol), refluxing for 48 h. An additional amount of **4c** (1.17 g, 9.45 mmol) was added after 24 h. The crude reaction residue was purified by crystallization from acetone; m.p. 180–181 °C; IR (cm^{-1}): 2 230 (CN), 1 640, 1 600, 1 510; UV (nm): λ_{max} 319, 285, 227, 204; ^{1}H-NMR ($CDCl_3$): δ 8.15 (d, 1H, *J* = 8.4, H-4), 7.99–7.90 (m, 3H, H-7 + H-2′ + H-6′), 7.93 (s, 1H, vinylic-H), 7.65 (dd, 1H, *J* = 8.2 and 1.2, H-6), 7.50 (dd, 1H, *J* = 8.2 and 1.2, H-5), 7.26 (dd, 2H, *J* = 8.8 and 8.4, H-3′ + H-5′); ^{13}C-NMR ($CDCl_3$ + DMSO-d_6): δ 164.55 (s), 159.54 (s), 143.69 (s), 137.88 (d), 130.45 (d), 130.27 (d), 129.67 (s), 127.39 (d), 125.31 (s), 123.43 (d), 116.17 (d), 114.77 (d), 114.32 (d), 111.98 (s, CN), 109.04 (d); MS *m/z* 264 (M^+). Anal. $C_{15}H_9FN_4$ (C, H, N, F).

*6.1.2.6. E-2-(2H-Benzotriazol-2-yl)-3-(4-fluorophenyl)acrylonitrile E-***6c**

In the same manner for *E*-**5c**, the title compound was obtained in 75% yield (1.25 g) after crystallization from diethyl ether starting from **3** (1 g, 6.3 mmol) and **4c** (1.17 g, 9.5 mmol); m.p. 195–196 °C; IR (cm^{-1}): 2 230 (CN), 1 610, 1 600, 1 550; UV (nm): λ_{max} 339, 277, 268, 223, 202; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6): δ 8.63 (s, 1H, vinylic-H), 8.08 (dd, 4H, *J* = 8.8 and 5.4, H-2′ + H-6′), 7.96–7.90 (m, 2H, H-4 + H-7), 7.54–7.46 (m, 2H, H-5 + H-6), 7.31 (dd, 2H, *J* = 8.8 and 8.6, H-3′ + H-5′); ^{13}C-NMR (DMSO-d_6): δ 166.38 (s), 161.37 (s), 144.32 (s), 136.83 (d), 132.64 (d), 132.46 (d), 128.53 (d, 2 CH), 128.37 (s), 126.90 (s), 118.04 (d, 2 CH), 116.76 (d), 116.32 (d), 113.03 (s, CN); MS *m/z* 264 (M^+). Anal. $C_{15}H_9FN_4$ (C, H, N, F).

*6.1.2.7. E-2-(1H-Benzotriazol-1-yl)-3-(4-chlorophenyl)acrylonitrile E-***5d**

This compound was prepared in 54.5% yield (2.24 g) from **2** (2.5 g, 15.8 mmol) and 4-chlorobenzaldheyde **4d** (2.5 g, 17.8 mmol) after reflux for 20 h and purification by column chromatography (eluent: diethyl ether–light petroleum 3:7) of the reaction residue; m.p. 145–146 °C (from acetone); IR (cm^{-1}): 2 230 (CN), 1 610, 1 590; UV (nm): λ_{max} 324, 289, 229, 205; ^{1}H-NMR ($CDCl_3$): δ 8.16 (d, 1H, *J* = 8.4, H-4), 7.95 (d, 1H, *J* = 8.4, H-7), 7.94 (s, 1H, vinylic-H), 7.88 (d, 2H, *J* = 8.6, H-2′ + H-6′), 7.66 (dd, 1H, *J* = 8.2 and 1.2, H-6), 7.52 (d, 2H, *J* = 8.6, H-3′ + H-5′), 7.47 (dd, 1H, *J* = 8.2 and 1.2, H-5); ^{13}C-NMR (DMSO-d_6): δ 145.39 (s), 139.06 (d), 136.28 (s), 131.33 (s), 131.17 (d, 2 CH), 129.53 (s), 129.29 (d), 129.24 (d, 2 CH), 125.36 (d), 119.94 (d), 113.68 (s, CN), 111.08 (d), 106.95 (s); MS *m/z* 280 (M^+). Anal. $C_{15}H_9ClN_4$ (C, H, N, Cl).

*6.1.2.8. E-2-(2H-Benzotriazol-2-yl)-3-(4-chlorophenyl)acrylonitrile E-***6d**

Starting from **3** (2.5 g, 15.8 mmol) and **4d** (2.5 g, 17.8 mmol) after reflux for 20 h and crystallization from acetone of the crude reaction residue, the title compound was obtained in 59% yield (2.03 g); m.p. 152–154 °C; IR (cm^{-1}): 2 240 (CN), 1 590, 1 580; UV (nm): λ_{max} 343, 280, 233, 202; ^{1}H-NMR ($CDCl_3$): δ 8.47 (s, 1H, vinylic-H), 7.94–7.89 (m, 4H, H-4 + H-7 + H-2′ + H-6′), 7.54–7.43 (m, 4H, H-5 + H-6 + H-3′ + H-5′); ^{13}C-NMR ($CDCl_3$): δ 145.05 (s), 138.03 (s), 135.44 (d), 131.08 (s), 131.03 (d, 2 CH), 129.62 (d, 2 CH), 128.70 (s), 128.26 (d, 2 CH), 118.24 (d, 2 CH), 112.68 (s, CN), 112.22 (s); MS *m/z* 280 (M^+). Anal. $C_{15}H_9ClN_4$ (C, H, N, Cl).

*6.1.2.9. E-2-(1H-Benzotriazol-1-yl)-3-(4-bromophenyl)acrylonitrile E-***5e**

This compound was prepared in 51.6% yield (2.1 g) from **2** (2 g, 12.6 mmol) and 4-bromobenzaldehyde **4e** (2.33 g, 12.6 mmol) after reflux for 30 h and purification by column chromatography on silica gel (eluent: diethyl ether–light petroleum 7:3) of the crude reaction residue; m.p. 130–131 °C (from diethyl ether); IR (cm^{-1}): 2 230 (CN), 1 590, 1 480; UV (nm): λ_{max} 325, 290, 229, 203;

^{1}H-NMR (DMSO-d_6): δ 8.16 (d, 1H, *J* = 8.4, H-4), 7.95 (d, 1H, *J* = 8.4, H-7), 7.92 (s, 1H, vinylic-H), 7.80 (d, 2H, *J* = 8.6, H-2′ + H-6′), 7.68 (d, 2H, *J* = 8.6, H-3′ + H-5′), 7.64 (dd, 1H, *J* = 8.2 and 1.8, H-6), 7.51 (dd, 1H, *J* = 8.2 and 1.8, H-5); ^{13}C-NMR ($CDCl_3$): δ 146.33 (s), 138.55 (d), 132.62 (d, 2 CH), 131.48 (s), 130.83 (d, 2 CH), 129.33 (s), 129.21 (d), 126.46 (s), 125.16 (d), 120.67 (d), 113.26 (s, CN), 110.18 (d), 107.09 (s); MS *m/z* 325 (M^+). Anal. $C_{15}H_9BrN_4$ (C, H, N, Br).

*6.1.2.10. E-2-(2H-Benzotriazol-2-yl)-3-(4-bromophenyl)acrylonitrile E-***6e**

In the same manner for *E*-**5e**, this compound was prepared in 61.5% yield (1.26 g) starting from **3** (1 g, 6.3 mmol) and **4e** (1.16 g, 6.3 mmol) after reflux for 22 h and purification by flash chromatography (eluent: diethyl ether–light petroleum 1:1) of the reaction residue; m.p. 137–138 °C (from diethyl ether); IR (cm^{-1}): 2 240 (CN), 1 600, 1 590; UV (nm): λ_{max} 344, 280, 235, 201; ^{1}H-NMR ($CDCl_3$): δ 8.63 (s, 1H, vinylic-H), 8.00–7.90 (m, 4H, H-4 + H-7 + H-2′ + H-6′), 7.72 (d, 2H, *J* = 8.2, H-3′ + H-5′), 7.60–7.40 (m, 2H, H-5 + H-6); ^{13}C-NMR ($CDCl_3$): δ 145.08 (s), 135.55 (d), 132.62 (d, 2 CH), 131.51 (s), 131.14 (d, 2 CH), 121.12 (s), 128.30 (d, 2 CH), 126.54 (s), 118.59 (s), 118.26 (d, 2 CH), 112.70 (s, CN); MS *m/z* 325 (M^+). Anal. $C_{15}H_9BrN_4$ (C, H, N, Br).

Further elution of the column gave a small amount of unreacted **3** (0.15 g, 15%) and 1*H*-benzotriazole **1** (0.16 g, 21%).

*6.1.2.11. (E/Z)-2-(1H-Benzotriazol-1-yl)-3-(4-trifluoromethylphenyl)acrylonitrile E-***5f** *and Z-***5f**

These compounds were prepared starting from **2** (2 g, 12.6 mmol) and 4-trifluoromethylbenzaldehyde **4f** (2.19 g, 12.6 mmol) after reflux for 48 h and purification by chromatography on silica gel column (eluent: diethyl ether–light petroleum 7:3). *E*-**5f** (0.62 g, 64.5%); m.p. 136–137 °C (from diethyl ether); IR (cm^{-1}): 2 240 (CN), 1 610; UV (nm): λ_{max} 320, 277, 205; ^{1}H-NMR (DMSO-d_6): δ 8.16 (d, 1H, *J* = 8.2, H-4), 8.06 (s, 1H, vinylic-H), 8.04 (d, 2H, *J* = 8.4, H-2′ + H-6′), 7.97 (d, 1H, *J* = 8.2, H-7), 7.80 (d, 2H, *J* = 8.4, H-3′ + H-5′), 7.67 (dd, 1H, *J* = 8.2 and 1.8, H-6), 7.51 (dd, 1H, *J* = 8.2 and 1.8, H-5); ^{13}C-NMR ($CDCl_3$): δ 146.44 (s), 137.36 (d), 133.88 (s), 133.30 (s), 132.67 (s), 131.41 (s), 129.72 (d, 2 CH), 129.45 (d), 126.26 (m, 2 CH), 125.36 (d), 120.80 (d), 112.92 (s, $^3J_{C-H}$ = 12.2 CN), 110.26 (d), 108.86 (s); MS *m/z* 314 (M^+). Anal. $C_{16}H_9F_3N_4$ (C, H, N, F).

Further elution of the column gave a mixture (0.51 g) of isomers *E*-**5f** and *Z*-**5f** in 2:1 ratio. This recrystallized from diethyl ether afforded an additional small amount of *E*-**5f** (0.14 g, 3.5%) and *Z*-**5f** (0.12 g, 3%); m.p. 110–112 °C; IR (cm^{-1}): 2 230 (CN), 1 635; UV (nm): λ_{max} 270, 206; ^{1}H-NMR ($CDCl_3$): δ 8.21 (d, 1H, *J* = 8.2, H-4), 7.74 (d, 2H, *J* = 8.2, H-2′ + H-6′), 7.60–7.46 (m, 2H, H-5 + H-6), 7.44 (d, 2H, *J* = 8.2, H-3′ + H-5′), 7.04 (d, 1H, *J* = 8.2, H-7), 6.03 (s, 1H, vinylic-H); ^{13}C-NMR ($CDCl_3$): δ 150.02 (s), 135.74 (s), 134.42 (s), 133.76 (s), 132.26 (s), 131.47 (s), 129.18 (d), 128.26 (d, 2 CH), 126.41 (m, 2 CH), 125.27 (d), 120.93 (d), 114.27 (s, $^3J_{C-H}$ = 0.0, CN), 110.87 (d), 94.12 (d); MS *m/z* 314 (M^+). Anal. $C_{16}H_9F_3N_4$ (C, H, N, F).

*6.1.2.12. E-2-(2H-Benzotriazol-2-yl)-3-(4-trifluoromethylphenyl)acrylonitrile E-***6f**

This compound was prepared starting from **3** (1 g, 6.3 mmol) and **4f** after 28 h under reflux and chromatography (eluent: mixtures of diethyl ether–light petroleum from 1:1 to 7:3) of the reaction residue; *E*-**6f** (1.48 g, 75%); m.p. 126–127 °C (from diethyl ether); IR (cm^{-1}): 2 240 (CN), 1 620, 1 570; UV (nm): λ_{max} 331, 272, 262, 220; ^{1}H-NMR (Me_2CO-d_6): δ 8.81 (s, 1H, vinylic-H), 8.35 (d, 2H, *J* = 8.2, H-2′ + H-6′), 8.10–7.90 (m, 4H, H-4 + H-7 + H-3′ + H-5′), 7.70–7.60 (m, 2H, H-5 + H-6); ^{13}C-NMR ($CDCl_3$): δ 145.18 (s), 134.73 (d, 2 CH), 133.65 (s), 133.33 (s), 132.68 (s), 129.97 (d, 2 CH), 128.54 (d, 2 CH), 126.20 (m, 2 CH), 120.76 (s), 118.33 (d, 2 CH), 114.15 (s), 112.32 (s, CN); MS *m/z* 314 (M^+). Anal. $C_{16}H_9F_3N_4$ (C, H, N, F).

*6.1.2.13. E-2-(1H-Benzotriazol-1-yl)-3-(4-carboxyphenyl)acrylonitrile E-***5g**

This compound was prepared in 19.6% yield starting from **2** (2 g, 12.6 mmol) and 4-carboxybenzaldheyde **4g** (1.9 g, 12.6 mmol) after reflux for 20 h followed by column chromatography (eluent: mixtures of diethyl ether–acetone with increasing percent of acetone) of the reaction residue. M.p. 259–260 °C (from acetone); IR (cm^{-1}): 3 250–3 000 (OH), 2 230 (CN), 1 700 (CO), 1 610, 1 570; UV (nm): λ_{max} 326, 287, 224 infl, 205; ^{1}H-NMR (DMSO-d_6): δ 8.38 (s, 1H, vinylic-H), 8.25 (d, 1H, *J* = 8.4, H-4), 8.15–8.04 (m, 5H, H-7 + 4 phenyl H), 7.78 (dd, 1H, *J* = 7.4 and 1.2, H-6), 7.60 (dd, 1H, *J* = 7.4 and 1.2, H-5), 3.50 (br s, 1H, OH); ^{13}C-NMR (DMSO-d_6): δ 166.66 (s, CO), 145.56 (s), 138.75 (d), 134.71 (s), 133.23 (s), 131.36 (s), 129.91 (d, 2 CH), 129.66 (d, 2 CH), 129.51 (d), 125.58 (d), 120.10 (d), 113.66 (s, CN), 111.34 (d), 108.30 (s); MS *m/z* 290 (M^+). Anal. $C_{16}H_{10}N_4O_2$ (C, H, N).

*6.1.2.14. E-2-(2H-Benzotriazol-2-yl)-3-(4-carboxyphenyl)acrylonitrile E-***6g**

In the same manner reported for *E*-**5g**, the title compound was obtained in 16.2% yield (0.59 g) starting from **3** (2 g, 12.6 mmol) and **4g** (1.9 g, 12.6 mmol); m.p.

277–279 °C; IR (cm^{-1}): 3 500–3 200 (OH), 2 240 (CN), 1 680 (CO), 1 610, 1 560, 1 510; UV (nm): λ_{max} 344, 277, 228 infl, 204; ^{1}H-NMR ($CDCl_3$): δ 10.15 (br s, H, OH), 8.64 (s, 1H, vinylic-H), 8.17 (d, 2H, *J* = 8.4, H-3′ + H-5′), 8.05 (d, 2H, *J* = 8.4, H-2′ + H-6′), 7.95–7.85 (m, 2H, H-4 + H-7), 7.53–7.40 (m, 2H, H-5 + H-6); ^{13}C-NMR (DMSO-d_6): δ 167.79 (s, CO), 144.37 (s), 137.00 (d), 136.85 (s), 132.98 (s), 129.80 (d, 2 CH), 129.63 (d, 2 CH), 128.65 (d, 2 CH), 127.90 (s), 118.05 (d, 2 CH), 112.80 (s, CN), 112.54 (s); MS *m/z* 290 (M^+). Anal. $C_{16}H_{10}N_4O_2$ (C, H, N).

*6.1.2.15. (E/Z)-2-(1H-Benzotriazol-1-yl)-3-(4-nitrophenyl)acrylonitrile E-***5h** *and Z-***5h**

These compounds were prepared from **2** (1 g, 6.3 mmol) and 4-nitrobenzaldheyde **4h** (1.10 g, 7.28 mmol) after refluxing for 20 h followed by column chromatography (eluent: diethyl ether–light petroleum 1:1). *E*-**5h** (0.79 g, 43.2%); m.p. 212–214 °C (from acetone); IR (cm^{-1}): 2 230 (CN), 1 620, 1 595; UV (nm): λ_{max} 332, 282, 203; ^{1}H-NMR (DMSO-d_6): δ 8.50–8.40 (m, 3H, vinylic-H + H-3′ + H-5′), 8.30–8.20 (m, 3H, H-4 + H-2′ + H-6′), 8.16 (d, 1H, *J* = 8.2, H-7), 7.78 (dd, 1H, *J* = 8 and 1.4, H-6), 7.60 (dd, 1H, *J* = 8 and 1.4, H-5); ^{13}C-NMR (DMSO-d_6): δ 148.54 (s), 145.68 (s), 142.18 (s), 137.28 (s), 136.67 (d), 131.25 (s), 130.74 (d, 2 CH), 129.65 (d), 125.75 (d), 124.18 (d, 2 CH), 120.17 (d), 113.28 (s, $^3J_{C-H}$ = 12.8, CN), 111.56 (d); MS *m/z* 291 (M^+). Anal. $C_{15}H_9N_5O_2$ (C, H, N).

Z-**5h** (0.30 g, 16.4%) (from acetone); m.p. 158–160 °C; IR (cm^{-1}): 2 230 (CN), 1 630, 1 600, 1 520; UV (nm): λ_{max} 290, 260, 205; ^{1}H-NMR (DMSO-d_6): δ 8.34 (d, 2H, *J* = 7.6, H-3′ + H-5′), 8.31 (d, 1H, *J* = 8.4, H-4), 7.71 (d, 2H, *J* = 7.6, H-2′ + H-6′), 7.69–7.50 (m, 2H, H-5 + H-6), 7.34 (d, 1H, *J* = 8.4, H-7), 7.18 (s, 1H, vinylic-H); ^{13}C-NMR (DMSO-d_6): δ 149.34 (s), 148.05 (s), 145.24 (s), 137.57 (s), 132.11 (s), 129.33 (d), 129.19 (d, 2 CH), 125.34 (d), 124.19 (d, 2 CH), 120.12 (d), 114.92 (s, $^3J_{C-H}$ = 0.0, CN), 111.26 (d), 98.42 (d); MS *m/z* 291 (M^+). Anal. $C_{15}H_9N_5O_2$ (C, H, N).

*6.1.2.16. (E/Z)-2-(2H-Benzotriazol-2-yl)-3-(4-nitrophenyl)acrylonitrile E-***6h** *and Z-***6h**

These compounds were prepared from **3** (1.5 g, 9.5 mmol) and **4h** (2.86 g, 19 mmol) after refluxing for 4 h followed by column chromatography (eluent: diethyl ether–light petroleum 1:1). *E*-**6h** (1.03 g, 37.3%): m.p. 224–226 °C (from acetone); IR (cm^{-1}): 2 230 (CN), 1 590, 1 560, 1 510; UV (nm): λ_{max} 348, 269, 210 sh, 204; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6): δ 8.83 (s, 1H, vinylic-H), 8.40 (d, 2H, *J* = 8.4, H-3′ + H-5′), 8.26 (d, 2H, *J* = 8.4, H-2′ + H-6′), 8.00–7.95 (m, 2H, H-4 + H-7), 7.57–7.52 (m, 2H, H-5 + H-6); ^{13}C-NMR (DMSO-d_6): δ 148.75 (s), 144.73 (s), 136.88 (s), 135.37 (d), 131.16 (d, 2 CH), 129.26 (d, 2 CH), 129.05 (s), 124.37 (d, 2 CH), 118.36 (d, 2 CH), 114.70 (s, $^3J_{C-H}$ = 13.3, CN), 112.71 (s); MS *m/z* 291 (M^+). Anal. $C_{15}H_9N_5O_2$ (C, H, N).

Z-**6h** (0.18 g, 6.5%): m.p. 157–159 °C (from acetone); IR (cm^{-1}): 2 220 (CN), 1 630, 1 600, 1 530; UV (nm): λ_{max} 289, 260 sh, 206; ^{1}H-NMR ($CDCl_3$): δ 8.32 (d, 2H, *J* = 8.4, H-3′ + H-5′), 8.23 (d, 1H, *J* = 8.2, H-4), 7.60–7.45 (m, 4H, H-5 + H-6 + H-2′ + H-6′), 7.07 (d, 1H, *J* = 8.2, H-7), 6.12 (s, 1H, vinylic-H), ^{13}C-NMR ($CDCl_3$): δ 149.87 (s), 149.19 (s), 146.11 (s), 138.02 (s), 132.09 (s), 129.33 (d), 128.94 (d, 2 CH), 125.42 (d), 124.47 (d, 2 CH), 120.98 (d), 114.06 (s, $^3J_{C-H}$ = 0.0, CN), 110.76 (d), 95.19 (d); MS *m/z* 291 (M^+). Anal. $C_{15}H_9N_5O_2$ (C, H, N).

*6.1.2.17. E-2-(1H-Benzotriazol-1-yl)-3-(4-aminophenyl)acrylonitrile E-***5i**

A suspension of *E*-**5h** (0.2 g, 0.69 mmol) and iron activated powder (0.38 g, 6.8 mmol) in ethanol (15 mL) was refluxed for 30 min when an aqueous solution of 2 N HCl (2 mL) and 50% ethanol (10 mL) was then added and the reflux continued for an additional 2 h. The unchanged iron was then filtered off, thoroughly washed with 50% ethanol solution, and the filtrate was evaporated in vacuo. The crude residue recrystallized from a 2:1 diethyl ether–acetone mixture, afforded *E*-**5i** (0.14 g, 78%); m.p. 177–179 °C; IR (cm^{-1}): 3 440 and 3 340 (NH_2), 2 200 (CN), 1 650, 1 620, 1 585, 1 550, 1 530; UV (nm): λ_{max} 388, 254, 203; ^{1}H-NMR (DMSO-d_6): δ 8.10 (d, 1H, *J* = 8.4, H-4), 7.82 (d, 1H, *J* = 8.4, H-7), 7.76 (d, 2H, *J* = 8.6, H-2′ + H-6′), 7.65 (s, 1H, vinylic-H), 7.60 (d, 1H, *J* = 8.4, H-6), 7.52 (dd, 1H, *J* = 8.4 and 1.2, H-5), 6.75 (d, 2H, *J* = 8.6, H-3′ + H-5′), 5.72 (br s, 2H, NH_2); MS *m/z* 261 (M^+). Anal. $C_{15}H_{11}N_5$ (C, H, N).

*6.1.2.18. E-2-(2H-Benzotriazol-2-yl)-3-(4-aminophenyl)acrylonitrile E-***6i**

Using the same procedure described for *E*-**5i**, the title compound was prepared in 57% yield (0.25 g) starting from *E*-**6h** (0.5 g, 1.7 mmol); m.p. 220–222 °C; IR (cm^{-1}): 3 450 and 3 360 (NH_2), 2 220 (CN), 1 630, 1 610, 1 590, 1 570, 1 520; UV (nm): λ_{max} 422, 298, 255, 204; ^{1}H-NMR ($CDCl_3$): δ 8.38 (s, 1H, vinylic-H), 7.95–7.85 (m, 2H, H-4 + H-7), 7.86 (d, 2H, *J* = 8.6, H-2′ + H-6′), 7.50–7.40 (m, 2H, H-5 + H-6), 6.74 (d, 2H, *J* = 8.6, H-3′ + H-5′), 4.26 (br s, 2H, NH_2); MS *m/z* 261 (M^+). Anal. $C_{15}H_{11}N_5$ (C, H, N).

6.1.2.19. 2-(1H-Benzotriazol-1-yl)acetic acid **7**

A solution of **2** (2 g, 12.6 mmol) in absolute ethanol (15 mL) was slowly added to a solution of Na (0.31 g, 13.5 mmol) in absolute ethanol (30 mL). After stirring at

room temperature for 15 min **4a** (1.34 g, 12.6 mmol) was added dropwise and the resulting mixture was refluxed for 4 h. The mixture was allowed to stand at room temperature and the formed precipitate was collected by filtration, obtaining the known **7** as sodium salt. This was dissolved with water and the solution was made acidic (pH = 2–3) with 2 N hydrochloric acid aqueous solution affording **7** (1.80 g, 80%) with m.p. 215–217 °C (literature [20]: m.p. 215–217 °C).

6.2. Pharmacology

Primary screening was conducted at 12.5 μg/mL against the virulent strain *Mycobacterium tuberculosis* H37Rv. *M. tuberculosis* was grown in BACTEC 12B medium containing radiolabelled substrate [25]. Labelled CO_2 produced was detected and quantified by the automatic BACTEC 460-radiometric system. Compounds effecting < 90% inhibition in the primary screening (MIC > 12.5 μg/mL) were not evaluated further. The standard compound used in this primary assay was rifampicin (MIC = 0.25 μg/mL). Compounds showing at least 90% inhibition in the primary screen are re-tested at lower concentration against *M. tuberculosis* H37Rv to determine the actual minimum inhibitory concentration (MIC) in a broth microdilution Alamar Blue assay (MABA) [26]. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. In addition these compounds were submitted to a primary screening of evaluation of their anti-*M. avium* activity in the BACTEC 460-radiometric system, using clarithromycin as a comparison (MIC = 2 μg/mL).

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