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# Synthesis and antimycobacterial activity of 3-aryl-, 3-cyclohexyl- and 3-heteroaryl-substituted-2-(1*H*(2*H*)-benzotriazol-1(2)-yl)prop-2-enenitriles, prop-2-enamides and propenoic acids. II

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**Abstract**

A series of 32 3-aryl-, 3-cyclohexyl-, and 3-heteroaryl-substituted-2-(1*H*(2*H*)-benzotriazol-1(2)-yl)-prop-2-enenitriles, prop-2-enamides and propenoic acids, was synthesized as a part of our research in the antitubercular field, according to an international program with the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF). This work reports the preparation and analytical and spectroscopic characterization (MS, UV, IR, $^1$H NMR) of all compounds synthesized. Among these only a few compounds (*E*-**4b**,**c**, *E*-**5a**, *E*-**7e** and *E*-**8d**) were found to be endowed with modest growth inhibition of *Mycobacterium tuberculosis*. However, the obtained results allowed to acquire interesting structure–activity relationships. 

*Keywords:* Antimycobacterial activity; 3-Substituted-2-(1*H*(2*H*)-benzotriazol-1(2)-yl)prop-2-enenitriles, prop-2-enamides, propenoic acids; SAR

## 1. Introduction

In spite of the therapeutic protocols used until the present, tuberculosis continues to represent one of the major threats to public health in the world [1–4]. The two main problems connected with this disease are the increasing emergence of multi-drug resistant (MDR) strains of *Mycobacterium tuberculosis* and synergy with the HIV infection [5,6]. The research of new prototypes, which may combat tuberculosis, is consequently, always actual. In this context, as a part of our studies concerning synthesis of biologically active benzotriazoles [7–10], we recently reported that several 3-aryl substituted 2-(benzotriazol-1(2)-yl)prop-2-enenitriles (Fig. 1) possess interesting antimycobacterial activity in vitro [11].

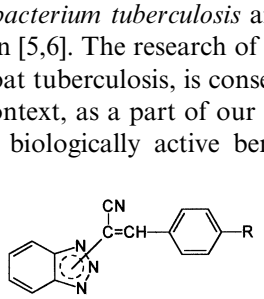


Fig. 1. General structure of antimycobacterial of 2-(benzotriazol-1(2)-yl)prop-2-enenitriles.

In particular, it has been pointed out that those compounds which have none or one electron-withdrawing substituent (R = H, Cl, Br, $CF_3$, $NO_2$) in the phenyl moiety exhibit the most significant activity (% inhibition growth between 50 and 98% at 12.5 μg ml$^{-1}$). Furthermore, the 1-benzotriazolyl derivatives seem generally to possess a greater activity than corresponding 2-benzotriazolyl derivatives. On the other hand the *E*-isomers resulted, in the few cases examined, more active than the *Z*-isomers. Starting from these results, in the present work we report the synthesis of a new series of compounds designed with the aim to increase antimycobacterial activity and acquire further information about the structure–activity relationships.

In particular, bearing in mind the high lipophilic properties of the mycobacterial cell wall [12], we have now investigated the effect on the activity deriving from the introduction in the phenyl ring of electron-releasing substituents (di- and tri-methoxy or 3,4-methylenedioxy)

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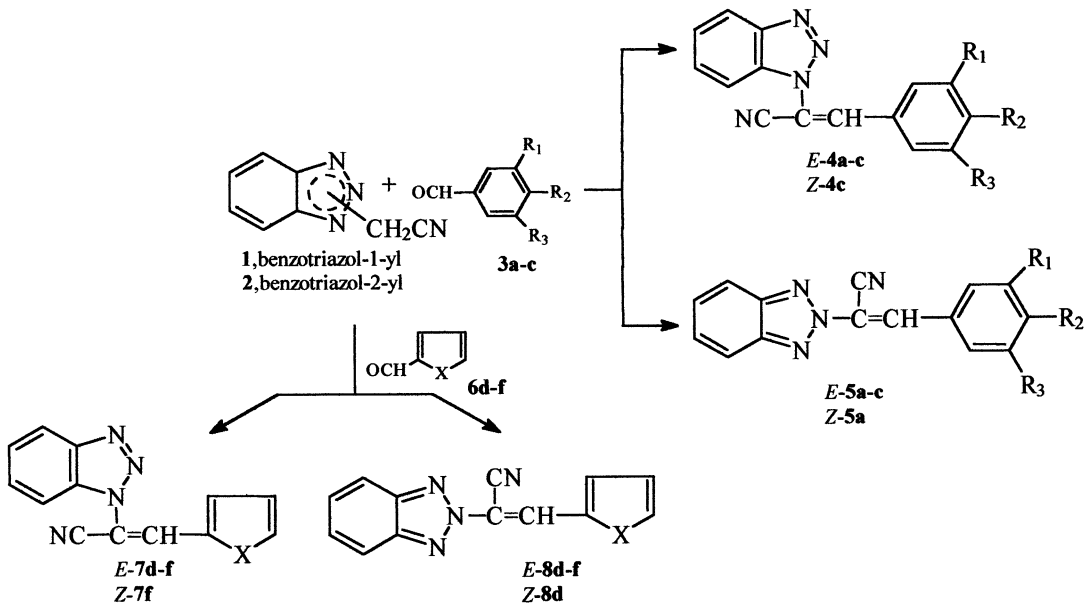



| Comp. | $R_1$ | $R_2$ | $R_3$ | X |
|---|---|---|---|---|
| a | $OCH_3$ | $OCH_3$ | | |
| b | $OCH_3$ | $OCH_3$ | $OCH_3$ | |
| c | $O–CH_2–O$ | | | |
| d | | | | NH |
| e | | | | O |
| f | | | | S |

Scheme 1.

more lipophilic than those previously reported (methyl and amino groups) [11]. In addition, the phenyl moiety was replaced with a cyclohexyl group in order to evaluate the effect of increased lipophilicity in the absence of the aromatic component. As a further development we also replaced the phenyl in the side chain with both a larger aromatic system (biphenyl and naphthyl) and a smaller electron-rich heterocycle (pyrrole, furane and thiophene) from which we would be able to evaluate the effect of the steric hindrance associated with the aromatic moiety.

Finally, in order to evaluate its importance, the cyano group was converted into a carboxamido- or carboxylic group. In this case the conversion interested only those compounds (*E*-**17a**–**e** and *E*-**18a**–**c**) of two previous series which resulted as possessing the best activity (% inhibition growth in the range 50–99% at 12.5 μg $ml^{-1}$) [11].

## 2. Chemistry

The preparation of the desired benzotriazolylprop-2-enenitriles **4**, **5**, **7** and **8**, depicted in Scheme 1, was accomplished by straightforward condensation (Knoevenagel condensation) of the known key intermediate benzotriazol-1(2)-acetonitriles (**1**) and (**2**) [11,13] with the appropriate commercial available aldehydes **3a**–**c** and **6d**–**f**, according to the method previously reported by us [11]. As previously observed, the *E*-isomer is generally obtained as the sole or the prevalent product. Whenever necessary, separation of the mixture of the two geometric isomers was easily performed by chromatography on a silica gel column.

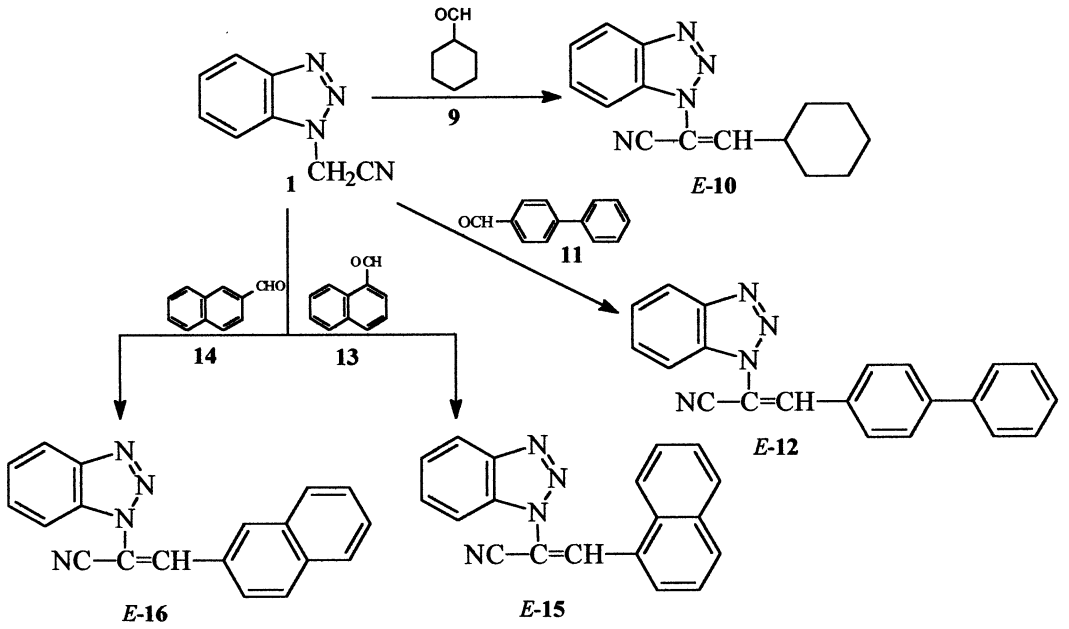
Synthesis of compounds *E*-**10**, *E*-**12**, *E*-**15** and *E*-**16**, was performed in the same conditions above described and is reported in Scheme 2. In this case only the *E*-isomers were obtained.

Conversion of the selected nitriles *E*-**17a**–**e** and *E*-**18a**–**c** into the corresponding carboxamides **19a**–**e** and **20a**–**c** was accomplished by hydrolysis with conc. $H_2SO_4$, while the same reaction carried out in conc. HCl on *E*-**17a**,**c**–**e** gave the acids *E*-**21a**,**c**–**e** (Scheme 3).

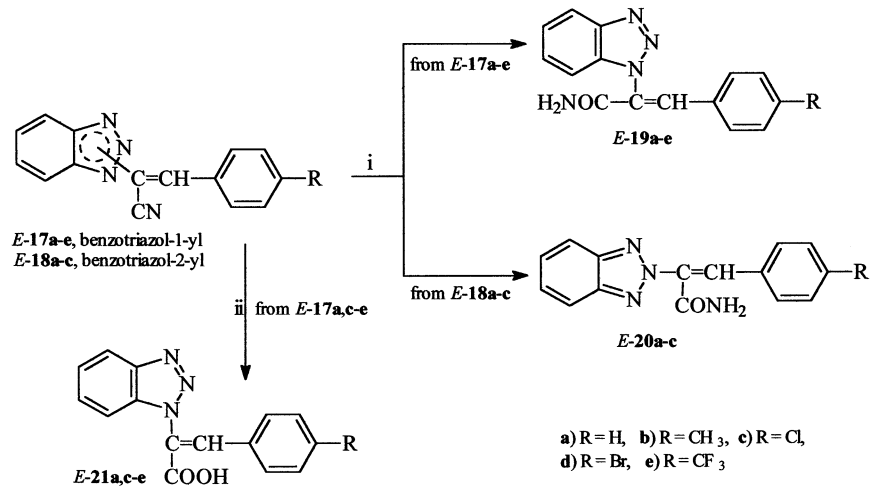
The chemical structure of all the new compounds was supported by their analytical (elemental analyses, MS) and spectral (IR, UV–Vis, $^1H$ NMR) data. In particular, the UV and NMR data were in accordance with those of the analogous compounds previously described by us [11].

## 3. Experimental

Melting points were determined by a Kofler hot stage or Digital Electrothermal apparatus, and are uncorrected. IR 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 ethanol with a Perkin–Elmer Lambda 5 spectrophotometer. The abbreviations used are as follows: sh (shoulder), infl (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 ($\delta$) 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. Thin-layer chromatography (TLC) was performed on precoated silica gel (0.25 mm) plates 60-$F_{254}$ Kieselgel from Merck. $R_f$, when reported, are determined using as eluent a mixture of diethyl ether–light petroleum 70:30. Column chromatography was performed using 230–400 mesh silica gel (Merck silica gel 60). Light petroleum refers to the fraction with b.p. 40–60 °C. Elemental analyses were performed by the Laboratorio di Microanalisi, Dipartimento di Scienze




Scheme 2.




Scheme 3.

Farmaceutiche, Università di Padova (Padua). The analytical results for C, H, N, and halogen, when present, were within ±0.4% of the theoretical values.

### 3.1. Intermediates

2-(Benzotriazol-1(2)-yl)acetonitriles **1** and **2** were prepared following the procedure previously described by us [11].

### 3.2. General procedure for preparation of 2-(1H-benzotriazol-1-yl)-3-arylprop-2-enenitriles (**4a**–**c**, **7d**–**f**, **10**, **12**, **15** and **16**) and 2-(2H-benzotriazol-2-yl)-3-arylprop-2-enenitriles (**5a**–**c**, and **8d**–**f**)

To a solution of the appropriate benzotriazolylacetonitrile **1** or **2** (6.3–12.6 mmol) and triethylamine (12.6–25.2 mmol) in toluene (25–30 ml) stirred at room temperature (r.t.) for 15–20 min, was added dropwise a solution of the required aryl-(**3a**–**c**, **11**, **13** and **14**), heteroaryl-(**6d**–**f**) or cyclohexane aldehyde (**9**) (6.3–21 mmol) in the same solvent (10 ml). After addition was complete, the whole mixture was heated under reflux for 6–72 h. The desired compounds, when not 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 diethyl ether–light petroleum 70:30) of the residue obtained after evaporation of the mother liquors. Analytical samples were recrystallized from a suitable solvent, as reported below. Yields, reaction conditions, melting point's (m.p.), analytical and spectroscopical data are reported as follows.

#### 3.2.1. E-2-(1H-benzotriazol-1-yl)-3-(3,4-dimethoxyphenyl)prop-2-enenitrile (E-**4a**)

This compound was obtained in 50% yield starting from **1** (2.5 g, 15.8 mmol) and 3,4-dimethoxybenzaldehyde (**3a**) (2.65 g, 15.9 mmol) after reflux for 20 h; m.p. 165–66 °C; IR (nujol): $\nu$ 2220 (CN), 1600, 1580 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 355, 298 infl, 249, 207 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 8.14 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-4), 7.89 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-7), 7.82 (s, 1H, vinyl-H), 7.69 (d, 1H, $J = 2.0$ Hz, H-2′), 7.63 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-6), 7.48 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-5), 7.43 (dd, 1H, $J = 8.2$ and 2.0 Hz, H-6′), 6.99 (d, 1H, $J = 8.2$ Hz, H-5′), 3.99 (s, 3H, $CH_3$), 3.98 (s, 3H, $CH_3$); MS: $m/z$ 306 ($M^+$). *Anal.* $C_{17}H_{14}N_4O_2$ (C, H, N).

#### 3.2.2. E-2-(1H-benzotriazol-1-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-enenitrile (E-**4b**)

This compound was obtained in 53.4% yield starting from **1** (2 g, 12.6 mmol) and 3,4,5-trimethoxybenzaldehyde (**3b**) (2.47 g, 12.6 mmol) after reflux for 44 h; m.p. 113–114 °C (from acetone–diethyl ether 1:2); IR (nujol): $\nu$ 2220 (CN), 1610, 1580 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 345, 240 sh, 205 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 8.16 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-4), 7.92 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-7), 7.85 (s, 1H, vinyl-H), 7.65 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-6), 7.53 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-5), 7.22 (s, 2H, H-2′ + H-6′), 3.96 (s, 9H, 3 $CH_3$); MS: $m/z$ 336 ($M^+$). *Anal.* $C_{18}H_{16}N_4O_3$ (C, H, N).

#### 3.2.3. E-2-(1H-benzotriazol-1-yl)-3-(3,4-methylenedioxyphenyl)prop-2-enenitrile (E-**4c**) and Z-2-(1H-benzotriazol-1-yl)-3-(3,4-methylenedioxyphenyl)prop-2-enenitrile (Z-**4c**)

An equimolar mixture of **1** (2 g, 12.6 mmol) and 3,4-(methylenedioxy)benzaldehyde (**3c**) (1.9 g, 12.6 mmol), was heated under reflux for 24 h. Then an extra portion of **3c** (1.9 g, 12.6 mmol) was added and the reflux continued for additional 24 h. Chromatography (eluent light petroleum–ethyl acetate 80:20) of the crude reaction residue gave.

*3.2.3.1. E-**4c**.* Yield 34%; m.p. 149–150 °C (from acetone); TLC (diethyl ether–light petroleum 70:30): $R_f$ 0.60; IR (nujol): $\nu$ 2220 (CN), 1720, 1620, 1600, 1590 $cm^{-1}$; UV (EtOH): $\lambda_{max}$: 357, 292 sh, 250, 204 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 8.14 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-4), 7.90 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-7), 7.80 (s, 1H, vinyl-H), 7.63 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-6), 7.61 (d, 1H, $J = 1.8$ Hz, H-2′), 7.49 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-5), 7.35 (dd, 1H, $J = 8.0$ and 1.8 Hz, H-6′), 6.94 (d, 1H, $J = 8.0$ Hz, H-5′), 6.11 (s, 2H, $CH_2$); MS: $m/z$ 290 ($M^+$). *Anal.* $C_{16}H_{10}N_4O_2$ (C, H, N).

*3.2.3.2. Z-**4c**.* Yield 3%; m.p. 98–100 °C (from acetone); TLC (diethyl ether–light petroleum 70:30): $R_f$ 0.27; IR (nujol): $\nu$ 2220 (CN), 1630, 1600 $cm^{-1}$; UV (EtOH): 346, 292, 251, 216 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 8.18 (dd, 1H, $J = 7.8$ and 1.8 Hz, H-4), 7.60–7.45 (m, 6H, H-5 + H-6 + H-7 + 3 phenyl-H), 6.69 (s, 1H, vinyl-H), 5.92 (s, 2H, $CH_2$); MS: $m/z$ 290 ($M^+$). *Anal.* $C_{16}H_{10}N_4O_2$ (C, H, N).

#### 3.2.4. E-2-(2H-benzotriazol-2-yl)-3-(3,4-dimethoxyphenyl)prop-2-enenitrile (E-**5a**) and Z-2-(2H-benzotriazol-2-yl)-3-(3,4-dimethoxyphenyl)-prop-2-enenitrile (Z-**5a**)

The title compounds were obtained starting from **2** (2.5 g, 15.8 mmol) and 3,4-dimethoxybenzaldehyde (**3a**) (2.65 g, 15.9 mmol) after reflux for 20 h. Chromatography (eluent light petroleum–ethyl acetate 70:30) of the crude reaction residue gave.

*3.2.4.1. E-**5a**.* Yield 46.9%; m.p. 166–167 °C (from acetone); TLC (diethyl ether–light petroleum 70:30): $R_f$ 0.58; IR (nujol): $\nu$ 2220 (CN), 1590, 1580, 1560 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 379, 332 infl, 284, 249, 204 nm; $^1H$

NMR ($CDCl_3$): $\delta$ 8.43 (s, 1H, vinyl-H), 7.93–7.88 (m, 2H, H-4 + H-7), 7.74 (d, 1H, $J = 2.2$ Hz, H-2′), 7.50–7.42 (m, 3H, H-5 + H-6 + H-6′), 6.97 (d, 1H, $J = 8.4$ Hz, H-5′), 3.99 (s, 3H, $CH_3$), 3.97 (s, 3H, $CH_3$); MS: $m/z$ 306 ($M^+$). *Anal.* $C_{17}H_{14}N_4O_2$ (C, H, N).

*3.2.4.2. Z-**5a***. Yield 3%; m.p. 233–235 °C (from acetone); TLC (diethyl ether–light petroleum 70:30): $R_f$ 0.25; IR (nujol): $\nu$ 2230 (CN), 1610, 1590, 1570 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 283, 243, 215 nm; $^1H$ NMR (DMSO-$d_6$): $\delta$ 8.10–8.07 (m, 2H, H-4 + H-7), 7.64–7.60 (m, 2H, H-5 + H-6), 7.19 (d, 1H, $J = 7.8$ Hz, H-6′), 6.88 (d, 1H, $J = 7.8$ Hz, H-5′), 6.85 (s, 1H, vinyl-H), 6.56 (s, 1H, H-2′), 3.71 (s, 3H, $CH_3$), 3.31 (s, 3H, $CH_3$); MS: $m/z$ 306 ($M^+$). *Anal.* $C_{17}H_{14}N_4O_2$ (C, H, N).

*3.2.5. E-2-(2H-benzotriazol-2-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-enenitrile (E-**5b**)*

This compound was obtained in 73% yield starting from **2** (1 g, 6.3 mmol) and 3,4,5-trimethoxybenzaldehyde (**3b**) (1.86 g, 9.48 mmol) after reflux for 44 h; m.p. 171–172 °C (from acetone); IR (nujol): $\nu$ 2240 (CN), 1610, 1580 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 368, 300 infl, 246, 203 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 8.45 (s, 1H, vinyl-H), 7.85–8.00 (m, 2H, H-4 + H-7), 7.40–7.50 (m, 2H, H-5 + H-6), 7.28 (s, 2H, H-2′ + H-6′), 3.96 (s, 9H, 3 $CH_3$); MS: $m/z$ 336 ($M^+$). *Anal.* $C_{18}H_{16}N_4O_3$ (C, H, N).

*3.2.6. E-2-(2H-benzotriazol-2-yl)-3-(3,4-methylenedioxyphenyl)prop-2-enenitrile (E-**5c**)*

This compound was obtained in 40% yield from **2** (1 g, 6.3 mmol) and 3,4-(methylenedioxy)benzaldehyde (**3c**) (1.9 g, 12.6 mmol) after reflux for 48 h; m.p. 244–245 °C; IR (nujol): $\nu$ 2240 (CN), 1620, 1590, 1570 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 378, 324, 252, 202 nm; $^1H$ NMR ($CDCl_3$ + DMSO-$d_6$): $\delta$ 8.52 (s, 1H, vinyl-H), 7.94–7.91 (m, 2H, H-4 + H-7), 7.87 (dd, 1H, $J = 8.0$ and 1.4 Hz, H-6′), 7.67 (d, 1H, $J = 1.4$ Hz, H-2′), 7.50–7.47 (m, 2H, H-5 + H-6), 7.00 (d, 1H, $J = 8.0$ Hz, H-5′), 6.16 (s, 2H, $CH_2$); MS: $m/z$ 290 ($M^+$). *Anal.* $C_{16}H_{10}N_4O_2$ (C, H, N).

*3.2.7. E-2-(1H-benzotriazol-1-yl)-3-(pyrrol-2-yl)prop-2-enenitrile (E-**7d**)*

This compound was obtained in 56% yield starting from **1** (2 g; 12.6 mmol) and 2-pyrrolecarboxaldehyde (**6d**) (2.4 g; 25.2 mmol) after reflux for 72 h; m.p. 183–184 °C (from diethyl ether); IR (nujol): $\nu$ 3200 (NH), 2220 (CN), 1610, 1590 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 361, 256 sh, 203, nm; $^1H$ NMR (DMSO-$d_6$): $\delta$ 11.79 (s, 1H, NH), 8.20 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-4), 7.96 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-7), 7.94 (s, 1H, vinyl-H), 7.73 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-6), 7.56 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-5), 7.33 (d, 1H, $J = 2.4$ Hz, H-3′) 7.28 (d, 1H, $J = 3.4$ Hz, H-5′), 6.47 (dd, 1H, $J = 3.4$ and 2.4 Hz, H-4′); MS: $m/z$ 235 ($M^+$). *Anal.* $C_{13}H_9N_5$ (C, H, N).

*3.2.8. E-2-(1H-benzotriazol-1-yl)-3-(2-furyl)prop-2-enenitrile (E-**7e**)*

This compound was obtained in 41% yield from **1** (2 g; 12.6 mmol) and 2-furaldehyde (**6e**) (1.15 g; 13.8 mmol) after reflux for 24 h; m.p. 137–138 °C; IR (nujol): $\nu$ 2210 (CN), 1625, 1605, 1580, 1560 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 324, 300 sh, 254, 206 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 8.14 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-4), 7.96 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-7), 7.78 (s, 1H, vinyl-H), 7.73 (d, 1H, $J = 1.8$ Hz, H-5′), 7.64 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-6), 7.48 (dd, 2H, $J = 8.4$ and 1.8 Hz, H-5), 7.17 (d, 1H, $J = 3.4$ Hz, H-3′), 6.70 (dd, 1H, $J = 3.4$ and 1.8 Hz, H-4′); MS: $m/z$ 236 ($M^+$). *Anal.* $C_{13}H_8N_4O$ (C, H, N).

*3.2.9. E-2-(1H-benzotriazol-1-yl)-3-(2-thienyl)-prop-2-enenitrile (E-**7f**) and Z-2-(1H-benzotriazol-1-yl)-3-(2-thienyl)prop-2-enenitrile (Z-**7f**)*

An equimolar mixture of **1** (2 g, 12.6 mmol) and 2-thiophenecarboxaldehyde (**6f**) (1.4 g, 12.6 mmol), was heated under reflux for 24 h. Then an extra portion of **6f** (0.7 g, 6.3 mmol) was added and the reflux continued for additional 6 h. Chromatography (eluent diethyl ether–light petroleum 70:30) of the crude reaction residue gave.

*3.2.9.1. E-**7f***. Yield 52.4%; m.p. 138–139 °C. TLC (diethyl ether–light petroleum 70:30): $R_f$ 0.61; IR (nujol): $\nu$ 2220 (CN), 1610, 1590 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 344, 283, 207 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 8.15 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-4), 8.11 (s, 1H, vinyl-H), 7.93 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-7), 7.76 (d, 1H, $J = 3.6$ Hz, H-5′), 7.71 (d, 1H, $J = 5.6$ Hz, H-3′), 7.65 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-6), 7.49 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-5), 7.24 (dd, 1H, $J = 5.6$ and 3.6 Hz, H-4′); MS: $m/z$ 252 ($M^+$). *Anal.* $C_{13}H_8N_4S$ (C, H, N).

*3.2.9.2. Z-**7f***. Yield 12.6%; m.p. 111–112 °C, (from diethyl ether); TLC (diethyl ether–light petroleum 70:30): $R_f$ 0.48; IR (nujol): $\nu$ 2220 (CN), 1620, 1610 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 326, 286, 260 infl, 206 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 8.20 (dd, 1H, $J = 7.4$ and 1.8 Hz, H-4), 7.89 (s, 1H, vinyl-H), 7.70–7.46 (m, 3H, H-5 + H-6 + H-7), 7.41 (d, 1H, $J = 5.0$ Hz, H-5′), 7.31 (d, 1H, $J = 3.8$ Hz, H-3′), 7.02 (dd, 1H, $J = 5.0$ and 3.8 Hz, H-4′); MS: $m/z$ 252 ($M^+$). *Anal.* $C_{13}H_8N_4S$ (C, H, N).

*3.2.10. E-2-(2H-benzotriazol-2-yl)-3-(pyrrol-2-yl)-prop-2-enenitrile (E-**8d**) and Z-2-(2H-benzotriazol-2-yl)-3-(pyrrol-2-yl)prop-2-enenitrile (Z-**8d**)*

An equimolar mixture of **2** (1 g, 6.3 mmol) and 2-pyrrolecarboxaldehyde (**6d**) (0.6 g, 6.3 mmol), was heated under reflux for 24 h. Then an extra portion of **6d** (0.6 g, 6.3 mmol) was added and the reflux continued for additional 24 h. Chromatography (eluent diethyl ether–light petroleum 70:30) of the crude reaction residue gave.

*3.2.10.1. E-**8d**.* Yield 73%; m.p. 203–205 °C (from acetone); TLC (diethyl ether–light petroleum 70:30): $R_f$ 0.53; IR (nujol): *v* 3400 (NH), 2240 (CN), 1625, 1615, 1570 $cm^{-1}$ UV (EtOH): $\lambda_{max}$ 398, 293, 286 infl, 202 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 11.68 (s, 1H, NH), 8.57 (s, 1H, vinyl-H), 7.91–7.46 (m, 2H, H-4 + H-7), 7.47–7.43 (m, 2H, H-5 + H-6), 7.32 (d, 1H, $J = 3.4$ Hz, H-5′), 7.19 (d, 1H, $J = 2.4$ Hz, H-3′), 6.43 (dd, 1H, $J = 3.4$ and 2.4 Hz, H-4′); MS: *m*/*z* 235 ($M^+$). *Anal.* $C_{13}H_9N_5$ (C, H, N).

*3.2.10.2. Z-**8d**.* Yield 9.4%; m.p. 139–140 °C (from diethyl ether); TLC (diethyl ether–light petroleum 70:30): $R_f$ 0.62; IR (nujol): *v* 2220 (CN), 1650, 1630, 1620, 1570 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 393, 287, 280 infl, 224 infl, 206 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 12.57 (s, 1H, NH), 7.99–7.94 (m, 2H, H-4 + H-7), 7.53–7.48 (m, 2H, H-5 + H-6), 7.27 (d, 1H, $J = 3.4$ Hz, H-5′), 7.13 (s, 1H, vinyl-H), 6.82 (d, 1H, $J = 2.4$ Hz, H-3′), 6.45 (dd, 1H, $J = 3.4$ and 2.4 Hz, H-4′); MS: *m*/*z* 235 ($M^+$). *Anal.* $C_{13}H_9N_5$ (C, H, N).

*3.2.11. E-2-(2H-benzotriazol-2-yl)-3-(2-furyl)prop-2-enenitrile (E-**8e**)*

This compound was obtained in 76% yield (1.12 g,) starting from **2** (1 g; 6.3 mmol) and 2-furaldehyde (**6d**) (0.7 g; 7.0 mmol) after 6 h under reflux; m.p. 137–138 °C (from light petroleum); IR (nujol): 2220 (CN), 1610, 1560 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 367, 280 sh, 248, 225, 203 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 8.30 (s, 1H, vinyl-H), 7.91–7.88 (m, 2H, H-4 + H-7), 7.73 (d, 1H, $J = 1.8$ Hz, H-5′), 7.50–7.40 (m, 2H, H-5 + H-6), 7.23 (d, 1H, $J = 3.8$ Hz, H-3′), 6.66 (dd, 1H, $J = 3.8$ and 1.8 Hz, H-4′); MS: *m*/*z* 236 ($M^+$). *Anal.* $C_{13}H_8N_4O$ (C, H, N).

*3.2.12. E-2-(2H-benzotriazol-2-yl)-3-(2-thienyl)-prop-2-enenitrile (E-**8f**)*

This compound was obtained in 88% yield starting from **2** (1 g; 6.3 mmol) and 2-thiophenecarboxaldehyde (**6f**) (0.7 g; 6.24 mmol) after 22 h under reflux; m.p. 174–176 °C (from diethyl ether); IR (nujol): 2240 (CN), 1615, 1570 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 368, 280, 226, 203 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 8.65 (s, 1H, vinyl-H), 7.93–7.88 (m, 2H, H-4 + H-7), 7.81 (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 + H-6), 7.25 (dd, 1H, $J = 4.8$ and 3.0 Hz, H-4′); MS: *m*/*z* 252 ($M^+$). *Anal.* $C_{13}H_8N_4S$ (C, H, N).

*3.2.13. E-2-(1H-benzotriazol-1-yl)-3-cyclohexylprop-2-enenitrile (E-**10**)*

This compound was obtained in 31% yield from **1** (1.50 g; 9.48 mmol) and cyclohexanecarboxaldehyde (**9**) (1.16 g; 10.3 mmol) after reflux for 24 h and chromatography (diethyl ether–light petroleum 1:1) of the crude reaction residue; m.p. 86–87 °C (from diethyl ether–hexane 1:1); IR (nujol): *v* 2230 (CN), 1630, 1610, 1590 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 294, 255, 216 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 8.12 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-4), 7.81 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-7), 7.61 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-6), 7.47 (dd, 2H, $J = 8.4$ and 1.8 Hz, H-5), 7.04 (d, 1H, $J = 10.6$ Hz, vinyl-H), 2.95–2.70 (m, 1H, H-1′), 2.00–1.70 (m, 6H, 3 $CH_2$), 1.50–1.15 (m, 4H, 2 $CH_2$); MS: *m*/*z* 252 ($M^+$). *Anal.* $C_{15}H_{16}N_4$ (C, H, N).

*3.2.14. E-2-(1H-benzotriazol-1-yl)-3-[(1,1′-biphenyl)-4-yl]prop-2-enenitrile (E-**12**)*

This compound was obtained in 37% yield from **1** (1.20 g; 7.59 mmol) and 4-biphenylcarboxaldehyde (**11**) (1.52 g; 8.34 mmol) after reflux for 20 h; m.p. 129–130 °C (from acetone); IR (nujol): *v* 2210 (CN), 1600 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 339, 292 infl, 236, 208 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 8.16 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-4), 8.06–7.90 (m, 3H, H-7 + H-2′ + H-6′), 7.77 (d, 2H, $J = 8.4$ Hz, H-3′ + H-5′), 7.72–7.60 (m, 3H, H-6 + H-2″ + H-6″), 7.56–7.40 (m, 5H, vinyl-H + H-5 + H-3″ + H-4″ + H-5″); MS: *m*/*z* 322 ($M^+$). *Anal.* $C_{21}H_{14}N_4$ (C, H, N).

*3.2.15. E-2-(1H-benzotriazol-1-yl)-3-(1-naphthyl)-prop-2-enenitrile (E-**15**)*

This compound was obtained in 36% yield from **1** (1.50 g; 9.48 mmol) and 1-naphthaldehyde (**13**) (1.50 g; 9.6 mmol) after reflux for 20 h; m.p. 137–138 °C (from acetone); IR (nujol): *v* 2225 (CN), 1610, 1590 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 347, 261, 220 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 8.73 (s, 1H, vinyl-H), 8.22 (dd, 1H, $J = 7.2$ and 1.8 Hz, H-4), 8.19 (dd, 1H, $J = 7.2$ and 1.8 Hz, H-7), 8.10–7.90 (m, 4H, H-6 + 3 aromatic-H), 7.74-7.58 (m, 4H, 4 aromatic-H), 7.52 (dd, 2H, $J = 7.2$ and 1.8 Hz, H-5); MS: *m*/*z* 296 ($M^+$). *Anal.* $C_{19}H_{12}N_4$ (C, H, N).

*3.2.16. E-2-(1H-benzotriazol-1-yl)-3-(2-naphthyl)-prop-2-enenitrile (E-**16**)*

This compound was obtained in 28% yield from **1** (1.50 g; 9.48 mmol) and 2-naphthaldehyde (**14**) (1.50 g; 9.6 mmol) after reflux for 20 h; m.p. 144–145 °C (from acetone); IR (nujol): *v* 2220 (CN), 1610 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 331, 273, 238, 215 nm; $^1H$ NMR ($CDCl_3$): $\delta$ 8.33 (s, 1H, H-1′), 8.17 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-4), 8.12 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-7), 8.10 (s, 1H, vinyl-H), 8.02–7.88 (m, 3H, H-6 + 2 aromatic-H), 7.77–7.45 (m, 5H, H-5 + 4 aromatic-H); MS: *m*/*z* 296 ($M^+$). *Anal.* $C_{19}H_{12}N_4$ (C, H, N).

*3.3. General procedure for preparation of amides (E-**19a–e**) and (E-**20a–c**)*

A suspension of the appropriate cyanoderivative *E*-**17a–e** or *E*-**18a–c** [11] (0.5 g; 1.54–2.03 mmol) in conc. sulfuric acid (10 ml) was stirred at 60 °C for 3 h. After cooling at r.t., the mixture was poured into 50 ml of crushed ice and stirring continued for an additional 30

min. The resulting precipitate was collected by filtration and purified by crystallization from a suitable solvent or by chromatography, as reported below.

*3.3.1. E-2-(1H-benzotriazol-1-yl)-3-phenylprop-2-enamide (E-**19a**)*

This compound was obtained in 50% yield; m.p. 149–150 °C (from acetone); IR (nujol): $\nu$ 3420 and 3280 ($NH_2$), 1670 (CO), 1630, 1620 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 307, 272, 205 nm; $^1H$ NMR ($CDCl_3$ + DMSO-$d_6$): $\delta$ 8.07 (dd, 1H, $J = 7.8$ and 1.8 Hz, H-4), 7.87 (s, 2H, $NH_2$), 7.79 (dd, 1H, $J = 7.8$ and 1.8 Hz, H-7), 7.73–7.38 (m, 7H, H-5 + H-6 + 5 phenyl H), 7.30 (s, 1H, vinyl-H); MS: $m/z$ 264 ($M^+$). *Anal.* $C_{15}H_{12}N_4O$ (C, H, N).

*3.3.2. E-2-(1H-benzotriazol-1-yl)-3-(4-methylphenyl)-prop-2-enamide (E-**19b**)*

This compound was obtained in 55% yield; m.p. 179–180 °C (from acetone); IR (nujol): $\nu$ 3380 and 3160 ($NH_2$), 1650 (CO), 1620, 1600 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 310, 276, 220 infl, 209 nm; $^1H$ NMR ($CDCl_3$ + DMSO-$d_6$): $\delta$ 8.06 (dd, 1H, $J = 8.0$ and 1.8 Hz, H-4), 7.71 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-7), 7.61 (s, 2H, $NH_2$), 7.60–7.38 (m, 2H, H-5 + H-6), 7.52 (d, 2H, $J = 8.2$ Hz, H-2′ + H6′), 7.25 (s, 1H, vinyl-H), 7.22 (d, 2H, $J = 8.2$ Hz, H-3′ + H-5′), 2.39 (s, 3H, $CH_3$); MS: $m/z$ 278 ($M^+$). *Anal.* $C_{16}H_{14}N_4O$ (C, H, N).

*3.3.3. E-2-(1H-benzotriazol-1-yl)-3-(4-chlorophenyl)-prop-2-enamide (E-**19c**)*

This compound was obtained in 89% yield; m.p. 191–192 °C (from acetone); IR (nujol): $\nu$ 3375 and 3170 ($NH_2$), 1650 (CO), 1620, 1600 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 311, 275, 204 nm; $^1H$ NMR ($CDCl_3$ + DMSO-$d_6$): $\delta$ 8.30 (s, 2H, $NH_2$), 8.10 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-4), 7.78 (dd, 1H, $J = 8.4$ and 1.8 Hz, H-7), 7.62 (m, 3H, H-6 + H-2′ + H-6′), 7.60–7.40 (m, 3H, H-5 + H-3′ + H-5′), 7.29 (s, 1H, vinyl-H); MS: $m/z$ 298/300 ($M^+$). *Anal.* $C_{15}H_{11}ClN_4O$ (C, H, Cl, N).

*3.3.4. E-2-(1H-benzotriazol-1-yl)-3-(4-bromophenyl)-prop-2-enamide (E-**19d**)*

This compound was obtained in 82% yield; m.p. 161–162 °C (from acetone–diethyl ether); IR (nujol): $\nu$ 3360 and 3200 ($NH_2$), 1670 (CO), 1600, 1580 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 309, 277, 206 nm; $^1H$ NMR (DMSO-$d_6$): $\delta$ 8.45 and 8.08 (2 s, 2H, $NH_2$), 8.22 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-4), 7.86 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-7), 7.76–7.50 (m, 6H, H-5 + H-6 + 4 phenyl H), 7.41 (s, 1H, vinyl-H); MS: $m/z$ 342/344 ($M^+$). *Anal.* $C_{15}H_{11}BrN_4O$ (C, H, Br, N).

*3.3.5. E-2-(1H-benzotriazol-1-yl)-3-(4-trifluoromethylphenyl)prop-2-enamide (E-**19e**)*

This compound was obtained in 79% yield; m.p. 193–194 °C (from acetone); IR (nujol): $\nu$ 3380 and 3290 ($NH_2$), 1690 (CO), 1650, 1620, 1600 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 311, 272, 206 nm; $^1H$ NMR (DMSO-$d_6$): $\delta$ 8.38 and 8.01 (2 s, 2H, $NH_2$), 8.12 (dd, 1H, $J = 7.6$ and 1.2 Hz, H-4), 8.02 (dd, 1H, $J = 7.6$ and 1.2 Hz, H-7), 7.82 (d, 2H, $J = 7.4$ Hz, H-3′ + H-5′), 7.74 (d, 2H, $J = 7.4$ Hz, H-2′ + H-6′), 7.63 (dd, 1H, $J = 7.6$ and 1.2 Hz, H-6), 7.49 (dd, 1H, $J = 7.6$ and 1.2 Hz, H-5), 7.43 (s, 1H, vinyl-H); MS: $m/z$ 332 ($M^+$). *Anal.* $C_{16}H_{11}F_3N_4O$ (C, H, N).

*3.3.6. E-2-(2H-benzotriazol-2-yl)-3-phenylprop-2-enamide (E-**20a**)*

This compound was obtained in 60% yield; m.p. 173–175 °C (from acetone); IR (nujol): $\nu$ 3480 and 3360 ($NH_2$), 1670 (CO), 1640, 1600 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 330, 260 sh, 225, 203 nm; $^1H$ NMR ($CDCl_3$ + DMSO-$d_6$): $\delta$ 7.99 (s, 2H, $NH_2$), 7.89 (m, 2H, H-4 + H-7), 7.79 (s, 1H, vinyl-H); 7.67 (d, 2H, $J = 6.6$ Hz, H-2′ + H-6′), 7.50–7.30 (m, 5H, H-5 + H-6 + 3 phenyl H); MS: $m/z$ 264 ($M^+$). *Anal.* $C_{15}H_{12}N_4O$ (C, H, N).

*3.3.7. E-3-(4-methylphenyl)-2-(2H-benzotriazol-2-yl)-prop-2-enamide (E-**20b**)*

This compound was obtained in 58% yield; m.p. 199–201 °C (from acetone); IR (nujol): $\nu$ 3380 and 3180 ($NH_2$), 1700 (CO), 1650, 1630 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 331, 270, 203 nm; $^1H$ NMR ($CDCl_3$ + DMSO-$d_6$): $\delta$ 8.34 and 6.72 (2 s, 2H, $NH_2$), 7.96–7.86 (m, 2H, H-4 + H-7), 7.71 (s, 1H, vinyl-H), 7.59 (d, 2H, $J = 7.4$ Hz, H-2′ + H-6′), 7.48–7.40 (m, 2H, H-5 + H-6), 7.24 (d, 2H, $J = 7.4$ Hz, H-3′ + H-5′), 2.36 (s, 3H, $CH_3$); MS: $m/z$ 278 ($M^+$). *Anal.* $C_{16}H_{14}N_4O$ (C, H, N).

*3.3.8. E-2-(2H-benzotriazol-2-yl)-3-(4-chlorophenyl)-prop-2-enamide (E-**20c**)*

This compound was obtained in 78% yield; m.p. 212–213 °C (from chloroform); IR (nujol): $\nu$ 3375 and 3170 ($NH_2$), 1650 (CO), 1630, 1560 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 330, 260, 227, 202 nm; $^1H$ NMR (DMSO-$d_6$): $\delta$ 8.50 and 8.14 (2 s, 2H, $NH_2$), 8.05 (m, 2H, H-4 + H-7), 7.82 (s, 1H, vinyl-H), 7.77 (d, 2H, $J = 8.4$ Hz, H-2′ + H-6′), 7.60–7.50 (m, 4H, H-5 + H-6 + H-3′ + H-5′); MS: $m/z$ 298/300 ($M^+$). *Anal.* $C_{15}H_{11}ClN_4O$ (C, H, Cl, N).

*3.4. General procedure for preparation of carboxylic acids (E-**21a**,**c**–**e**)*

A suspension of the appropriate cyanoderivative *E*-**17a**,**c**–**e** [11] (0.5 g; 1.54–2.03 mmol) in conc. hydrochloric acid aqueous (aq.) solution (20 ml) was stirred at 100–110 °C for 3 h. After cooling, the reaction mixture was diluted with iced-water (50 ml) and the resulting precipitate was collected by filtration, thoroughly washed with water and dried. All the obtained

compounds were purified by crystallization from a suitable solvent as reported below.

*3.4.1. E-2-(1H-benzotriazol-1-yl)-3-phenylpropenoic acid (E-**21a**)*

This compound was obtained in 48% yield; m.p. 210–212 °C (from acetone); IR (nujol): $\nu$ 1680 (CO), 1640, 1600 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 267, 207 nm; $^1H$ NMR (acetone-$d_6$): $\delta$ 8.34 (s, 1H, vinyl-H), 8.13 (dd, 1H, $J = 8.0$ and 1.6 Hz, H-4), 7.60–7.40 (m, 4H, H-6 + 3 phenyl H), 7.32 (dd, 1H, $J = 8.0$ and 1.6 Hz, H-7), 7.22 (dd, 1H, $J = 8.0$ and 1.6 Hz, H-5), 6.93 (d, 2H, $J = 8.0$ Hz, H-2′ + H-6′), 4.72 (br s, 1H, OH); MS: $m/z$ 265 ($M^+$). *Anal.* $C_{15}H_{11}N_3O_2$ (C, H, N).

*3.4.2. E-2-(1H-benzotriazol-1-yl)-3-(4-chlorophenyl)-propenoic acid (E-**21c**)*

This compound was obtained in 81% yield; m.p. 284–286 °C (from acetone); IR (nujol): $\nu$ 1710 (CO), 1640, 1590 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 273, 210 nm; $^1H$ NMR (acetone-$d_6$): $\delta$ 8.33 (s, 1H, vinyl-H), 8.13 (dd, 1H, $J = 7.8$ and 1.6 Hz, H-4), 7.58–7.42 (m, 3H, H-5 + H-6 + H-7), 7.28 (d, 2H, $J = 8.4$ Hz, H-3′ + H-5′), 6.95 (d, 2H, $J = 8.4$ Hz, H-2′ + H-6′), 4.78 (br s, 1H, OH); MS: $m/z$ 299/301 ($M^+$). *Anal.* $C_{15}H_{10}ClN_3O_2$ (C, H, Cl, N).

*3.4.3. E-2-(1H-benzotriazol-1-yl)-3-(4-bromophenyl)-propenoic acid (E-**21d**)*

This compound was obtained in 23% yield, together with the previously described amide *E*-**19d** (31% yield). Separation of two compounds was accomplished by treatment of the crude product with 10% $NaHCO_3$, filtration of the insoluble *E*-**19d** and reprecipitation of *E*-**21d** from the alkaline solution by acidification with 2N HCl; m.p. 278–279 °C (from acetone); IR (nujol): $\nu$ 1690 (CO), 1640, 1590 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 273, 207 nm; $^1H$ NMR (acetone-$d_6$): $\delta$ 8.32 (s, 1H, vinyl-H), 8.13 (dd, 1H, $J = 7.8$ and 1.6 Hz, H-4), 7.60–7.40 (m, 3H, H-5 + H-6 + H-7), 7.29 (d, 2H, $J = 8.4$ Hz, H-3′ + H-5′), 6.94 (d, 2H, $J = 8.4$ Hz, H-2′ + H-6′), 4.86 (br s, 1H, OH); MS: $m/z$ 343/345 ($M^+$). *Anal.* $C_{15}H_{10}BrN_3O_2$ (C, H, Br, N).

*3.4.4. E-2-(1H-benzotriazol-1-yl)-3-(4-trifluoromethyl-phenyl)propenoic acid (E-**21e**)*

The title compound was obtained in 19% yield together with the previously described amide *E*-**19e** (38% yield). Separation of two compounds was accomplished by treatment of the crude product with 10% $NaHCO_3$, filtration of the insoluble *E*-**19e** and reprecipitation of *E*-**21e** from the alkaline solution by acidification with 2 N HCl; m.p. 252–253 °C (from acetone); IR (nujol): $\nu$ 1700 (CO), 1640 $cm^{-1}$; UV (EtOH): $\lambda_{max}$ 264, 208 nm; $^1H$ NMR (acetone-$d_6$): $\delta$ 8.42 (s, 1H, vinyl-H), 8.13 (dd, 1H, $J = 8.2$ and 1.8 Hz, H-4), 7.70–7.40 (m, 5H, H-5 + H-6 + H-7 + H-3′ + H-5′), 7.18 (d, 2H, $J = 8.4$ Hz, H-2′ + H-6′), 4.70 (br s, 1H, OH); MS: $m/z$ 333 ($M^+$). *Anal.* $C_{16}H_{10}F_3N_3O_2$ (C, H, N).

*3.5. Antimycobacterial assay*

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 and Coordinating Facility (TAACF) screening program for the discovery of novel drugs for the 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*.

Primary screening was conducted, respectively, at 12.5 μg $ml^{-1}$ for *E*-**4a**–**c**, *E*-**5a**–**c**, *E*-**7d**–**f**, *Z*-**7f**, *E*-**8d**–**f**, *E*-**19a**–**c**, *E*-**20a**–**c**, and 6.25 μg $ml^{-1}$ in the case of compounds *E*-**10**, *E*-**12**, *E*-**15**, *E*-**16**, *E*-**19d**,**e**, and *E*-**21a**,**c**–**e**, against the virulent strain *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [14]. Compounds exhibiting fluorescence were tested in the Bactec 460 radiometric system [14]. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Compounds effecting $< 90\%$ inhibition in the primary screening (MIC $> 6.25$ μg $ml^{-1}$) were not evaluated further. The standard compound used in this primary assay was rifampicin (MIC = 0.25 μg $ml^{-1}$).

## 4. Results and discussion

Compounds *E*-**4a**–**c**, *E*-**5a**–**c**, *E*-**7d**–**f**, *Z*-**7f**, *E*-**8d**–**f**, *E*-**10**, *E*-**12**, *E*-**15**, *E*-**16**, *E*-**19a**–**e**, *E*-**20a**–**c**, *E*-**21a**,**c**–**e**, were tested in vitro for antitubercular activity against *M. tuberculosis* H37Rv in comparison with Rifampicin as reference drug. Results of this primary screening are reported in Table 1.

Since, none of the tested compounds exhibited in the primary screening a % growth inhibition $> 90\%$, the evaluation was not continued further. It appears from Table 1 that the compounds of this second series resulted much less active than those previously reported [11]. Replacement of electron-withdrawing substituents in C-4 of the phenyl ring with two or more electron-releasing groups (compounds **4** and **5**), or of the phenyl moiety with cyclohexyl or larger aromatic rings (*E*-**10**, *E*-**12**, *E*-**15** and *E*-**16**) produced a general strong reduction of the activity in spite of the increased lipophilic character of molecules, thus indicating that the steric hindrance as well as the kind of the substituent may play a relevant role in the activity. On the other hand,

Table 1
In vitro evaluation of antitubercular activity as % growth inhibition at 12.5 μg $ml^{-1}$ concentration (rifampicin MIC = 0.25 μg $ml^{-1}$) versus *M. tuberculosis*

| Comp. | Inhibition growth (%) | Comp. | Inhibition (%) | Comp. | Inhibition (%) |
|---|---|---|---|---|---|
| *E*-**4a** | 0 | *E*-**8d** | 35 | *E*-**19d** [a] | 0 |
| *E*-**4b** | 43 | *E*-**8e** | 9 | *E*-**19e** [a] | 0 |
| *E*-**4c** | 41 | *E*-**8f** | 10 | *E*-**20a** | 0 |
| *E*-**5a** | 31 | *E*-**10** [a] | 0 | *E*-**20b** | 0 |
| *E*-**5b** | 0 | *E*-**12** [a] | 0 | *E*-**20c** | 0 |
| *E*-**5c** | 0 | *E*-**15** [a] | 10 | *E*-**21a** [a] | 0 |
| *E*-**7d** | 14 | *E*-**16** [a] | 0 | *E*-**21c** [a] | 0 |
| *E*-**7e** | 33 | *E*-**19a** | 0 | *E*-**21d** [a] | 0 |
| *E*-**7f** | 6 | *E*-**19b** | 0 | *E*-**21e** [a] | 0 |
| *Z*-**7f** | 0 | *E*-**19c** | 0 | | |

[a] Tested at 6.25 μg $ml^{-1}$.

similar behavior was observed when the phenyl ring was replaced with an electron-rich heterocycle (compounds **7** and **8**), thus confirming that an electron-withdrawing effect in the aryl moiety is necessary for the activity. Furthermore, data reported in Table 1 are in agreement with our previous observations that the 1-benzotriazolyl derivatives (**4**) are more active than 2-isomers (**5**). Finally, conversion of the cyano group into a carboxamido (*E*-**19** and *E*-**20**) or carboxylic (*E*-**21**) group produced the loss of the activity, thus indicating that an increase of the hydrophilic properties is not profitable for antimycobacterial activity.

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