European Journal of Medicinal Chemistry 46 (2011) 320–326

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and anti-mycobacterial activities of triazoloquinolones

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ARTICLE INFO

Article history:
Received 16 July 2010
Received in revised form
24 September 2010
Accepted 11 November 2010
Available online 19 November 2010

Keywords:
Triazoloquinolones
Benzotriazolo
M. tuberculosis
Anti-mycobacterial activity

ABSTRACT

A number of quinolone derivatives have been reported to possess anti-mycobacterial activity. Generally. *Mycobacterium tuberculosis* isolates expressing resistance to both isoniazid and rifampin are susceptible to fluoroquinolones. Benzotriazole is a hetero-bicyclic aromatic ring endowed with interesting chemical and biological properties and pharmacological activities. In a preliminary study we have recently reported the activity of triazolo[4,5-*h*]quinolone-carboxylic acids, a new class of benzotriazole derivatives active against multi-drug resistant *M. tuberculosis* (MDR-*Mtb*). In this study we confirm that this novel class of quinolones is endowed with a selective anti-mycobacterial activity, coupled with absence of cytotoxicity.

The SAR analysis of the new derivatives in comparison with the previous series shows that the methyl group is the most effective substituent in both N-3 and N-9 positions of the ring system.

1. Introduction

Benzo[*d*][1,2,3]triazole, here named benzotriazole, has been extensively investigated by Katritzky et al. as a synthetic auxiliary for it behaves as good leaving group after reaction with a variety of carbonyl group [1–3], whereas Sparatore and Pagani have studied the biological properties [4,5] and described the interesting pharmacological activities of several benzotriazole derivatives bearing substituents at positions 1 or 2 [6–14]. Furthermore, our group has developed a separate chemistry and further investigated the biological potential of benzotriazoles [15–19]. In particular, we described several 3-aryl-2-benzotriazol-(2)-yl-acrylonitriles which were found to be endowed with antitubercular activity [20–23], partly retained by the corresponding amides and carboxylic acids [21], and 2-benzotriazol-(1)-yl-3-phenylacrylonitriles, which possess an interesting anticancer activity (Fig. 1) [22,23].

Furthermore, by connection of either *f* or *h* sides of the quinoline ring and both the adjacent carbon atoms of the triazole the angular *N*-tricyclic systems triazolo[4,5-*f*]quinoline (**2**) and triazolo[4,5-*h*]quinoline (**3**), were also obtained (Fig. 2).

Triazoloquinoline (**2**) was first reported by German Authors in 1934 [24], but not mentioned in Chemical Abstracts, while the isomer derivative **3** was reported as a side product during the preparation of 7,8-triazoloquinolin-5-arsonic acid by Slater in 1932 [25] and recently obtained by Ferlin et al. through an alternative route [26].

Several derivatives of **2** and **3** endowed with biological properties have been reported in the literature over the last three decades. Among them, carboxylic acid derivatives of triazolo[4,5-*f*]quinolines (**2a**) and triazolo[4,5-*h*]quinolines (**3a**), depicted in Fig. 3, showed antimicrobial activity [27,28], whereas some 9-aminoalkylaminotriazolo[4,5-*f*]quinolines (**2b**) exhibited anticancer activity [29].

The linear derivative triazolo[4,5-*g*]quinoline and its 5-Cl-derivative were obtained by our group in 2000 [30]. As summarized in Fig. 4, further substituted triazolo[4,5-*g*]quinolines (**4**) were later synthesized [31–33]. In particular, 9-dihydrotriazolo[4,5-*g*]quinoline-1-oxide (**4a**) stood out as a new small molecule endowed with a selective, promising activity in cell-based assays against HIV-1wt and clinically relevant NNRTI resistant mutants [34].

Concerning angular triazolo[4,5-*h*]quinoline derivatives (**3**), Fig. 5 summarized the structural formula of 3,9-disubstituted-6-oxo-6,9-dihydrotriazolo[4,5-*h*]quinolone-carboxylic acids and their esters (**3b**) which were recently reported by our group as a new class of quinolones possessing a potent anti-mycobacterial activity. On the contrary, triazolo[4,5-*f*]quinolines (**2a**) were completely inactive. The triazolo[4,5-*h*]quinilines (**3b**) turned out to be particularly interesting for their activity against MDR-*Mtb*, several derivatives exhibiting MIC values in the range of 0.5–3.2 μg/mL [35,36].

Recently the derivative 3,9-dimethyl-6-oxo-6,9-dihydrotriazolo[4,5-*h*]quinoline-7-carboxylic acid (**3c**) has been selected for further

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doi:10.1016/j.ejmech.2010.11.020

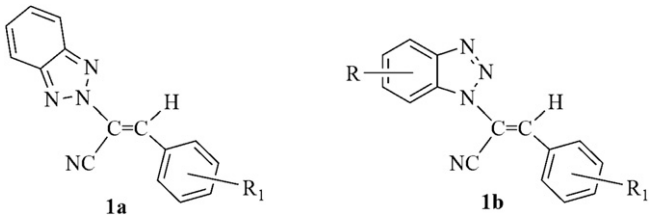


Fig. 1. Benzotriazolyl-phenylacrylonitriles endowed with antitubercular (**1a**) or antiproliferative (**1b**) activities.

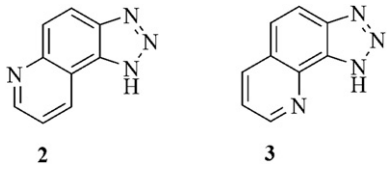


Fig. 2. Triazolo[4,5-*f*]quinoline (**2**) and triazolo[4,5-*h*]quinoline (**3**).

experiments. When determined by the twofold broth or agar dilution method, the MIC against mycobacteria, including *Mycobacterium tuberculosis*, was 0.5–2 μg/mL. To test the intracellular activity, human macrophages J774-A1 were infected with *M. tuberculosis* H37Rv and successively grown in the absence or presence of **3c** at 2.0 and 1.0 μg/mL. After 7 days the macrophages were lysed and the growth of mycobacteria determined by plate counting. In treated cultures, the number of viable mycobacteria was 5×10^3 and 8×10^3 CFU/mL, at 1.0 and 2.0 μg/mL, respectively, while in the untreated controls the number of mycobacteria was 1×10^6 CFU/mL.

In the aim to extend the SAR analysis for this class of compounds, we have now designed, synthesized and submitted to biological evaluation two novel series of triazolo[4,5-*f*]quinolone carboxylic acids (**11c**–**f** and **11g**–**j**), and their ethyl ester parents as more lipophilic pro-drugs (**10c**–**f** and **10g**–**j** respectively). In the first series we have kept the best subtituents of the previous series in N_9 (methyl, ethyl, allyl and *tert*-butyl) [35] and replaced the methyl with a benzyl in N_3 (**11c**–**f**) in order to evaluate the biological effect of increased steric bulk of this substituent in this position.

In the second series we have kept the methyl group in N_3 and introduced on N_9 a further series of substituents (2-methylallyl, cyclopropylmethyl, cyclobutylmethyl and 2,4-difluorobenzyl) (**11g**–**j**) in order to evaluate the biological effect of both increased steric bulk and lipophilia of these substituents in this position.

All the new triazolo[4,5-*f*]quinolone carboxylic acids (**11c**–**j**) were tested against *M. tuberculosis* H37Rv, *M. tuberculosis* H37Ra, *Mycobacterium smegmatis* and *Mycobacterium bovis*. Moreover, to investigate the spectrum of antibacterial activity, all compounds were tested against a panel of gram-positive bacteria including enterococci (*Enterococcus faecalis*), staphylococci (*Staphylococcus aureus*, *Staphylococcus epidermidis*), gram-negative bacteria including *Acinetobacter baumanii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and against *Candida* sp. The most promising compounds (**11c**, **11g** and the selected **3c**) were evaluated in cell-based assays for cytotoxicity in human hematological tumors cell cultures (MT-4).

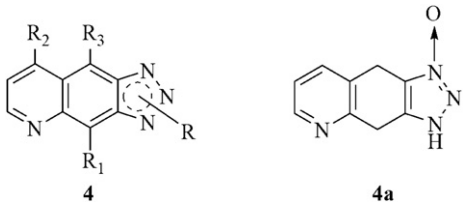


Fig. 4. Triazolo[4,5-*g*]quinoline derivatives.

2. Chemistry

The synthetic route which led to 9-alkyl-3-benzyl-6-oxo-6,9-dihydrotriazole[4,5-*h*]quinoline-7-carboxylic ethyl esters and to the acids (**10c**–**j** and **11c**–**j** respectively) is depicted in Schemes 1–3.

Alkylation of 4-nitrobenzotriazole in acetonitrile with benzyl bromide using NaOH as base was previously described by Diehl and Kendall [37]. In the cited patent, the Authors claimed that the reaction of 4-nitrobenzotriazole with an unspecified benzyl halide in the same conditions yielded only two of three possible isomers, namely 1-benzyl-4-nitrobenzotriazole (**6a**) and 2-benzyl-4-nitrobenzotriazole (**6b**), but not the 1-benzyl-7-nitrobenzotriazole (**6c**) reported in Scheme 1. The two isomers were isolated by means of their different solubility in acidic media. No data relating to structure elucidation and characterization of the two compounds were reported with the exception of melting points.

Following the same procedure a routine thin layer chromatography (Hexane/Ethyl acetate 7/3) conversely showed three different spots, instead of the two expected, at R_f values of 0.68, 0.65, 0.63. Consequently we were able to isolate via column chromatography three isomeric benzyl benzotriazoles which were fully identified and characterized by NMR spectroscopy using a previously reported methodology applied for the corresponding alkyl derivatives [14,17].

By ^{13}C NMR spectroscopy, we were able to assign structure **6b** to the isomer with $R_f = 0.65$ which showed a characteristic signal at 61 ppm for the benzyl carbon while the other two isolated compounds showed for the same carbon signals at higher fields (55 and 53 ppm). In fact when a side alkyl chain is bound to 2-nitrogen of the benzotriazole ring, the atom electron withdrawing effect of the two adjacent nitrogen atoms causes a carbon chemical shift at lower fields compared to the corresponding 1- or 3- alkyl derivatives. Furthermore, the differences in the methylene carbon chemical shifts could also arise from the steric interactions between the benzyl methylene and proximate aromatic proton in **6a** and methylene proton and the nitro group in **6c** which could shift the chemical shift of methylene carbons in these compounds relative to that in **6b**.

The assignment of the correct structure to the compounds with $R_f = 0.68$ and 0.63 was achieved by NOE experiments. Compound having $R_f = 0.63$ was identified as 1-benzyl-4-nitrobenzotriazole (**6a**)

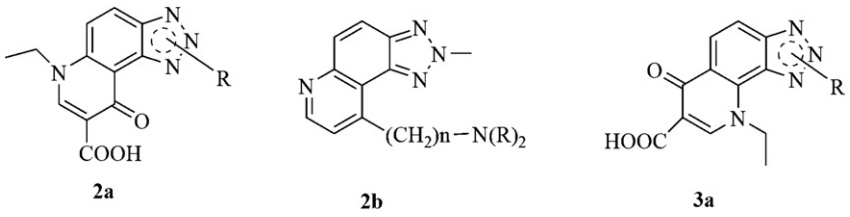


Fig. 3. Triazolo[4,5-*f*]quinolines (**2**) and triazolo[4,5-*h*]quinolines (**3**) endowed with antimicrobial and anticancer activities.

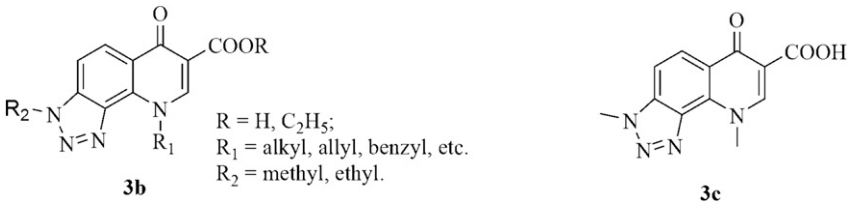
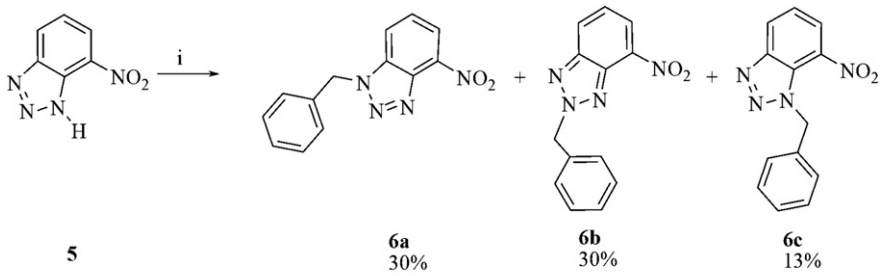


Fig. 5. Triazolo[4,5-*f*]quinolone derivatives endowed with potent anti-mycobacterial activity.



Conditions: i) $BrCH_2C_6H_5$, NaOH 2N, 85 °C, 30 h;

Scheme 1. Synthesis of *N*-benzyl-4(7)-nitrobenzotriazoles (**6a**–**c**).

in that it showed a positive NOE effect on H-7 when the singlet at 5.96 ppm was irradiated. No positive NOE effect was instead observed for compound with $R_f = 0.68$ when the benzyl methylene at 6.27 ppm was irradiated, according to the structure of compounds **6c**.

As reported in Scheme 2, hydrogenation of **6a** by hydrazine and palladium/charcoal under refluxing ethanol afforded the amino-derivative (**7**) in good yield, which according to Gould–Jacobs reaction with diethyl ethoxymethylenemalonate in Dowtherm gave the aminomethylenemalonate derivative (**8**) in 56% yield. Thermal ring closure of **8** at 250 °C in Dowtherm gave the corresponding quinolone (**9a**) in 55% yield.

The reactions reported in Scheme 3 describe the synthesis of the desired 3,9-disubstituted-6-oxo-6,9-dihydro-triazolo[4,5-*h*]quinoline-7-carboxylic acids (**11c**–**j**).

Ethyl 3-benzyl-6-oxo-6,9-dihydro-triazolo[4,5-*h*]quinoline-7-carboxylate (**9a**) and ethyl 3-methyl-6-oxo-6,9-dihydro-triazolo [4,5-*h*]quinoline-7-carboxylate **(9b)** underwent alkylation at N-9 position with the appropriate alkyl bromide in the presence of KOH or NaH to give the corresponding 9-substituted-triazolo[4,5-*h*] quinolone esters (**10c**–**j**) in 8–76% yields accompanied by their corresponding acids (**11c**–**j**) in 7–75%. 3-Methyl-intermediate (**9b**) was in turn prepared according to the pathway previous reported [35,36].

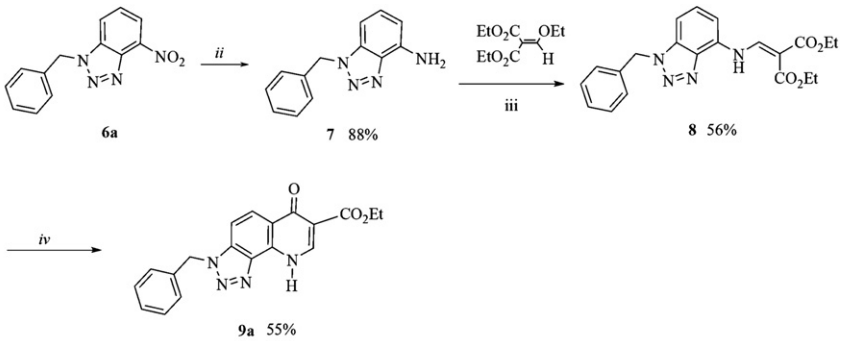
All the acids (**11c**–**j**) were also obtained in 90–98% yields by alkaline hydrolysis of the esters (**10c**–**j**).

3. Results and discussion

The activity (MIC: μg/mL) of all the new derivatives (esters **10c**–**j** and acids **11c**–**j**) and **3c** against *M. tuberculosis* H37Rv, *M. tuberculosis* H37Ra and other mycobacteria (*M. smegmatis* and *M. bovis*), is reported in Table 1

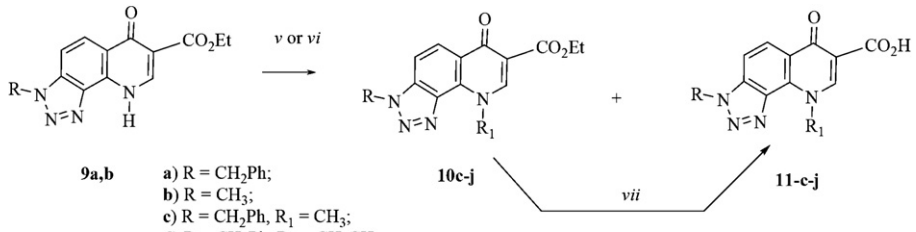
Comparison of the activity of our compounds with reference drugs such as ethambutol, rifampin, streptomycin and ciprofloxacin, showed that only compounds (**11c** and **11g**) were active with MICs in the range 4–32 μg/mL, whereas all other compounds (acids and esters) displayed limited or no activity. Only compound **3c** had activity comparable to reference drugs.

When MICs were determined against a panel of gram-positive and gram-negative bacteria and against *Candida* sp. all compounds (**10c**–**j**, **11c**–**j** and **3c**) were inactive (MIC > 64 μg/mL).



Conditions: ii) NH_2NH_2, Pd/C 10 %, ethanol, 78 °C, 3h; iii) Dowtherm, 150 °C, 7h; iv) Dowtherm, 250 °C, 15 min.

Scheme 2. Synthesis of the key intermediate 3-benzyl-6-oxo-6,9-dihydro-]triazolo[4,5-*h*]quinoline-7-carboxylate (**9**).



Conditions: v) R_1-Br, KOH pellets, DMSO, rt, 1 h; vi) R_1-Br, NaH, DMF, 90 °C, 8 h, vii) NaOH, H_2O, 100 °C, 2 h.

Scheme 3. Synthesis of 3,9-disubstituted-6-oxo-6,9-dihydro-triazolo[4,5-*h*]quinoline-7-carboxylic acids (**11c**–**j**).

Table 1
In vitro evaluation (MIC: μg/mL) of compounds (**10c**–**j** and **11c**–**j**) against mycobacteria.

Comp	*M. tuberculosis* H37Rv	*M. tuberculosis* H37Ra	*M. smegmatis* mc^2155	*M. bovis* BCG
10c	64	>128	>128	64
10d	64	>128	>128	>128
10e	64	>128	>128	64
10f	64	32	>128	64
10g	32	4	16	8
10h	64	>128	>128	>128
10i	64	>128	>128	64
10j	64	>128	>128	64
11c	8	4	32	4
11d	64	8	64	16
11e	64	4	>128	8
11f	64	64	>128	64
11g	16	8	32	16
11h	64	32	64	32
11i	32	16	64	16
11j	32	16	64	16
3c	0.5	0.5	5	4
Ethambutol	5	4	0.5	2
Rifampin	<0.5	≤0.06	8	–
Streptomycin	1	1	–	–
Ciprofloxacin	0.5	0.5	0.125	0.25

As far as the cytotoxicity is concerned, the tested compounds (**11c**, **11g** and **3c**) showed $CC_{50} > 100$ μg/mL against MT-4 cells.

Overall, the several favourable characteristics of the most promising compound **3c**, which include activity against mycobacteria (particularly *M. tuberculosis*) comparable to reference compounds, good *in vitro* therapeutic index obtained comparing the cytotoxicity [35,36] and the anti-mycobacterial activity, and the very narrow spectrum of antibacterial activity, directed only against mycobacteria, are supportive of further evaluation.

4. Conclusion

In summary, our experiments confirm that 3,9-disubstituted-6-oxo-6,9-dihydrotriazolo[4,5-*h*]quinolone-carboxylic acids are in general endowed with anti-mycobacterial activity, whereas their ethyl ester parents are not active *in vitro*. The SAR analysis of the new derivatives in comparison with the previous series shows that the methyl group is the most effective substituent in N-3 position of the ring system and, very interestingly, and conversely to known quinolones, the methyl group is the best substituent also in the N-9 position.

The spectrum of antibacterial activity demonstrated by this new class of quinolones resulted very narrow, suggesting a specific anti-mycobacterial potential. This characteristic is quite relevant in the context of a new anti-mycobacterial drug for two main reasons. First, the limited spectrum shall not disturb the existing normal flora and, therefore, will likely be better tolerated as compared with wide spectrum existing treatments such as rifamycins and quinolones. Second, the presence of a reduced number of susceptible organisms will limit the selection of resistant mutants among species that are not specifically targeted by the treatment, therefore reducing the risk of vertical transmission of resistance across species.

These interesting results, supported also by the good cytotoxicity profile, suggest to further characterize compound **3c**, currently selected as lead compound, and to continue the chemical and biological exploration of this new class of potential anti-mycobacterial agents.

5. Experimental

5.1. Chemistry

Melting points were measured with a Digital Electrothermal IA9100 melting points apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian XL-200 (200 MHz) spectrometer. Solutions were typically prepared in either deuterochloroform ($CDCl_3$) or deuterated dimethyl sulfoxide (DMSO-d_6) with chemical shifts referenced to tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm. MS were recorded using combined HP 5790-HP 5970 GC/MS apparatus. Column chromatography was performed using 70–230 mesh (Merck silica gel 60). The R_f values and the progress of the reactions and the product purity were checked by TLC using Merck F-254 commercial plates. Analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values.

5.1.1. Starting materials

All chemicals were obtained from commercially available sources (Aldrich) and used without purification. 4-Nitrobenzotriazole (**5**) and ethyl 3-methyl-6-oxo-6,9-dihydro-triazolo[4,5-*h*]quinoline-7-carboxylate were prepared according to literature [24,35,36].

5.1.2. Preparation of 1(2)-benzyl-4-nitrobenzotriazole (*6a*,*b*) and 1-benzyl-7-nitrobenzotriazole (*6c*)

To a solution of 4-nitrobenzotriazole (**5**) (89.5 mmol) in acetonitrile (440 mL) and NaOH (pellets) (1.5 eq,) benzyl bromide

(89.5 mmol) was slowly added. The resulting mixture was then warmed to 85 °C and stirred overnight. The crude precipitate was then collected by filtration and purified by flash chromatography (hexane/ethyl acetate 7/3) to separate the three isomers **6a**–**c**. Yields, melting points, analytical and spectroscopical data are reported below.

*5.1.2.1. 1-Benzyl-4-nitrobenzotriazole (**6a**).* This compound was obtained in 30% yield, TLC (Hexane/Ethyl acetate 7/3): R_f 0.63, mp 129–130 °C (ethanol). ^{1}H NMR ($CDCl_3$): δ 8.24 (d, 1H, J = 8.8, H-5), 7.73 (d, 1H, J = 8.2 Hz, H-7), 7.55 (dd, 1H, J = 8.8 and 8.4 Hz, H-6), 7.40–7.34 (m, 5 phenyl-H), 5.96 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 141.47 (Cq), 138.77 (Cq), 134.97 (Cq), 133.61 (Cq), 129.17 (2CH), 128.89 (CH), 127.52 (2CH), 126.66 (CH), 121.20 (CH), 116.94 (CH), 52.92 (CH_2). MS m/z 254 (M^+). Anal. Calcd for $C_{13}H_{10}N_4O_2$: C, 61.41; H, 3.96; N, 22.04. Found C, 61.73; H, 4.19; N, 21.87.

*5.1.2.2. 1-Benzyl-7-nitrobenzotriazole (**6c**).* This compound was obtained in 13% yield, TLC (Hexane/Ethyl acetate 7/3): R_f 0.68, mp 82–84 °C (ethanol). ^{1}H NMR ($CDCl_3$): δ 8.44 (d, 1H, J = 8.2, H-6), 8.23 (d, 1H, J = 7.8 Hz, H-4), 7.50 (dd, 1H, J = 8.2 and 7.8 Hz, H-5), 7.26–7.09 (m, 5 phenyl-H), 6.27 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 149.50 (Cq), 135.34 (Cq), 135.10 (Cq), 133.65 (Cq), 128.84 (2CH), 128.21 (CH), 127.30 (CH), 127.15 (2CH), 125.27 (CH), 123.25 (CH), 55.26 (CH_2). MS m/z 254 (M^+). Anal. Calcd for $C_{13}H_{10}N_4O_2$: C, 61.41; H, 3.96; N, 22.04. Found C, 61.73; H, 4.19; N, 21.87.

*5.1.2.3. 2-Benzyl-4-nitrobenzotriazole (**6b**).* This compound was obtained in 30% yield, TLC (Hexane/Ethyl acetate 7/3): R_f 0.65, mp 101–102 °C (ethanol). ^{1}H NMR ($CDCl_3$): δ 8.40 (d, 1H, J = 8.0 Hz, H-5), 8.26 (d, 1H, J = 8.2 Hz, H-7), 7.57–7.36 (m, 6H, 5 phenyl-H + H-6), 6.01 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 146.71 (Cq), 137.97 (Cq), 136.65 (Cq), 133.66 (Cq), 128.93 (3CH), 128.58 (2CH), 126.23 (CH), 124.91 (CH), 124.52 (CH), 61.01 (CH_2). MS m/z 254 (M^+). Anal. Calcd for $C_{13}H_{10}N_4O_2$: C, 61.41; H, 3.96; N, 22.04. Found C, 61.19; H, 4.03; N, 21.91.

*5.1.3. Preparation of 1-benzyl-4-aminobenzotriazole (**7**)*

A suspension of compound **6a** (1.0 g, 3.9 mmol), 10% palladium/charcoal (0.1 g) and hydrazine monohydrate (0.5 mL) in ethanol (15 mL) was refluxed under stirring for 1 h. The reaction mixture was then cooled at room temperature, the catalyst filtered off, and the solvent and the excess of hydrazine removed *in vacuo*. The crude product was used without any further purification.

Yield 88%, mp 92–94 °C (diethyl ether). ^{1}H NMR ($CDCl_3$ + DMSO): δ 7.30 (m, 5 phenyl-H), 7.12 (dd, 1H, J = 8.0 and 7.8 Hz, H-6), 6.73 (d, 1H, J = 8.0 Hz, H-5), 6.40 (d, 1H, J = 7.8 Hz, H-7), 5.97 (s, 2H, NH_2), 5.81 (s, 2H, CH_2); MS m/z 224 (M^+). Anal. Calcd for $C_{13}H_{12}N_4$: C, 69.62; H, 5.39; N, 24.98. Found C, 69.29; H, 5.60; N, 24.79.

*5.1.4. Preparation of diethyl 2-((1-benzyl-benzotriazol-4-ylamino) methylene)malonate (**8**)*

To a stirred solution of **7** (0.86 g, 3.8 mmol) in 10 mL of Dowtherm warmed at 60 °C, diethyl ethoxymethylenemalonate (1.27 g, 5.9 mmol) was added dropwise. After then, the mixture was heated up to 150 °C and stirred for 7 h. After cooling at room temperature, the mixture was poured over 90 mL of hexane and stirred for 1 h vigorously. The solid obtained was used without any further purification.

Yield 56%, mp 133–135 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 11.64 (d, 1H, J = 13.8 Hz, NH), 9.51 (d, 1H, J = 13.8 Hz, NH–C*H*=C), 7.37–7.20 (m, 6H, H-6 + 5 phenyl-H), 7.07–6.97 (m, 2H, H-5 + H-7), 5.85 (s, 2H, C*H*$_2$–Ph), 4.43–4.25 (m, 4H, 2O–C*H*$_2$–CH$_3$), 1.46–1.34 (m, 6H, 2O–CH2–C*H*$_3$). MS m/z 394 (M^+). Anal. Calcd for $C_{21}H_{22}N_4O_4$: C, 63.95; H, 5.62; N, 14.20. Found C, 64.30; H, 5.41; N, 14.56.

*5.1.5. Preparation of ethyl 3-benzyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylate (**9a**)*

A suspension of aminomethylenemalonate derivative **8** (2.74 g, 6.95 mmol) in 30 mL of hot Dowtherm (250 °C) was vigorously stirred for 15 min. The reaction mixture was then cooled at room temperature and poured into 250 mL of hexane and stirred for 2 h. The crude solid obtained collected by filtration and purified by chromatography ($CHCl_3$/MeOH 9.5:0.5) gave the title compound as a solid.

Yield 55%, mp 255–257 °C (ethanol). ^{1}H NMR ($CDCl_3$ + DMSO): δ 13.27 (s, 1H, NH), 8.57 (s, 1H, H-8), 8.33 (d, 1H, J = 8.8 Hz, H-5), 7.54 (d, 1H, J = 8.8 Hz, H-4), 7.34 (m, 5 phenyl-H), 5.98 (s, 2H, C*H*$_2$–Ph), 4.32 (q, 2H, J = 7.4, O–C*H*$_2$–CH$_3$), 1.37 (t, 3H, J = 7.4, O–CH$_2$–C*H*$_3$). MS m/z 348 (M^+). Anal. Calcd for $C_{19}H_{16}N_4O_3$: C, 65.51; H, 4.63; N, 16.08. Found C, 65.22; H, 4.70; N, 16.37.

*5.1.6. Preparation of 3,9-disubstituted-6-oxo-6,9-dihydro-triazolo [4,5-h]quinoline-7-carboxylic esters and acids (**10c**–**j** and **11c**–**j**)*

To a suspension of 10 mmol of finely powered KOH (method **v**) or of NaH (50% oil suspension) (method **vi**) in DMSO (6 mL), 3.0 mmol of ethyl 3-benzyl(or methyl)-quinoline-7-carboxylate derivative **9a** (or **9b**) was added and left stirring at rt for 15 min. Then the appropriate alkyl bromide (3.5 mmol) was added and the stirring was continued at rt for 1 h (method **v**) or at 90 °C for 8 h (method **vi**). On cooling the reaction mixture was added of 40 mL of water. The precipitate when formed was filtered while the mother liquors were extracted with chloroform (3 × 50 mL) and the combined extracts were dried on anhydrous sodium sulphate and evaporated *in vacuo*. The crude solids obtained, corresponding to the 9-alkylated-esters (**10c**–**j**), were crystallized by ethanol. The mother liquors acidified by 2N HCl afforded a further precipitate corresponding to the acids: **11c** (34%); **11d** (75%); **11e** (16%); **11f** (17%); **11g** (7%); **11h** (17%); **11i** (14%); **11j** (12%). All the acids (**11c**–**j**) were also obtained in 90–98% yields by hydrolysis of the esters (**10c**–**j**) in the presence of 2N NaOH heating at 100 °C for 2 h (method **vii**). Method of preparation, yields, melting points, analytical and spectroscopical data are reported below.

*5.1.6.1. Ethyl 3-benzyl-9-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylate (**10c**).* Method (**v**): yield 53%, mp 228–230 °C. ^{1}H NMR ($CDCl_3$ + DMSO): δ 8.57 (s, 1H, H-8), 8.52 (d, 1H, J = 9.0 Hz, H-5), 7.47 (d, 1H, J = 9.0 Hz, H-4), 7.34 (m, 5 phenyl-H), 5.94 (s, 2H, C*H*$_2$Ph), 4.65 (s, 3H, N–C*H*$_3$), 4.38 (q, 2H, J = 7.0 Hz, O–C*H*$_2$CH$_3$), δ 1.41 (t, 3H, J = 7.0 Hz, OCH$_2$C*H*$_3$). MS m/z 362 (M^+). Anal. Calcd for $C_{20}H_{18}N_4O_3$: C, 66.29; H, 5.01; N, 15.46. Found C, 65.92; H, 5.23; N, 15.29.

*5.1.6.2. 3-Benzyl-9-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylic acid (**11c**).* Method (**v**): yield 34%, method (**vii**): yield 94%, mp 265–267 °C. ^{1}H NMR ($CDCl_3$ + DMSO): δ 15.45 (s, 1H, COO*H*), 9.12 (s, 1H, H-8), 8.43 (d, 1H, J = 8.8 Hz, H-5), 8.05 (d, 1H, J = 8.8, H-4), 7.36 (m, 5 phenyl-H), 6.12 (s, 2H, C*H*$_2$–Ph), 4.72 (s, 3H, N–C*H*$_3$). MS m/z 334 (M^+). Anal. Calcd for $C_{18}H_{14}N_4O_3$: C, 64.66; H, 4.22; N, 16.76. Found C, 64.40; H, 4.59; N, 16.51.

*5.1.6.3. Ethyl 3-benzyl-9-ethyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylate (**10d**).* Method (**v**): yield 8%, mp 161–163 °C. ^{1}H NMR (DMSO): δ 8.61 (s, 1H, H-8), 8.51 (d, 1H, J = 9.0 Hz, H-5), 7.64 (d, 1H, J = 9.0 Hz, H-4), 7.40–7.20 (m, 5 phenyl-H), 5.98 (s, 2H, C*H*$_2$Ph), δ 5.32 (q, 2H, J = 7.2, N–C*H*$_2$–CH$_3$), 4.42 (q, 2H, J = 7.0 Hz, O–C*H*$_2$–CH$_3$), 1.71–1.41 (m, 6H, 2CH$_3$). MS m/z 376 (M^+). Anal. Calcd for $C_{21}H_{20}N_4O_3$: C, 67.01; H, 5.36; N, 14.88. Found C, 67.36; H, 5.22; N, 15.10.

*5.1.6.4. 3-Benzyl-9-ethyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylic acid (**11d**).* Method (**v**): yield 75%, method (**vii**):

yield 98%, mp 210–212 °C. ^{1}H NMR ($CDCl_3$ + DMSO): δ 15.29 (s, 1H, COO*H*), 8.92 (s, 1H, H-8), 8.54 (d, 1H, *J* = 8.8 Hz, H-5), 7.64 (d, 1H, *J* = 8.8, H-4), 7.36 (m, 5 phenyl-H), 5.98 (s, 2H, C*H*$_2$–Ph), 4.36 (q, 2H, *J* = 7.2 Hz, N–C*H*$_2$–CH$_3$), 1.705 (t, 3H, *J* = 7.2 Hz, N–CH$_2$–C*H*$_3$). MS *m*/*z* 348 (M^+). Anal. Calcd for $C_{19}H_{16}N_4O_3$: C, 65.51; H, 4.63; N, 16.08. Found C, 65.85; H, 4.44; N, 16.31.

*5.1.6.5. Ethyl 9-allyl-3-benzyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylate (**10e**).* Method (**vi**): yield 25%, mp 183–185 °C. ^{1}H NMR ($CDCl_3$): δ 9.02 (s, 1H, H-8), 7.78 (d, 1H, *J* = 9.0 Hz, H-5), 7.47 (d, 1H, *J* = 9.0 Hz, H-4), 7.40–7.20 (m, 5 phenyl-H), 5.98 (s, 2H, C*H*$_2$Ph), 5.80 (m, 1H, CH$_2$–C*H*=CH$_2$), 5.20 (d, 2H, *J* = 9.0 Hz, CH$_2$–CH=C*H*$_2$), 5.00 (d, 2H, *J* = 9.0 Hz, C*H*$_2$–CH=CH$_2$), 4.20 (q, 2H, *J* = 7.0 Hz, O–C*H*$_2$–CH$_3$), 1.29 (t, 3H, *J* = 7.0 Hz, OCH$_2$C*H*$_3$). MS *m*/*z* 388 (M^+). Anal. Calcd for $C_{22}H_{20}N_4O_3$: C, 68.03; H, 5.19; N, 14.42. Found C, 68.31; H, 5.01; N, 14.22.

*5.1.6.6. 9-Allyl-3-benzyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylic acid (**11e**).* Method (**vi**): yield 16%, method (**vii**): yield 90%, mp 216–218 °C. ^{1}H NMR (DMSO): δ 15.48 (s, 1H, COOH), 9.12 (s, 1H, H-8), 8.45 (d, 1H, *J* = 9.2 Hz, H-5), 8.13 (d, 1H, *J* = 8.6 Hz, H-4), 7.38 (m, 5 phenyl-H), 6.40–6.05 (m, 3H, N–C*H*$_2$–CH=CH$_2$ + N–CH$_2$–C*H*=CH$_2$), 5.96 (s, 2H, C*H*$_2$Ph), 5.23–5.08 (m, 2H, CH$_2$–CH=C*H*$_2$). MS *m*/*z* 360 (M^+). Anal. Calcd for $C_{20}H_{16}N_4O_3$: C, 66.66; H, 4.48; N, 15.55. Found C, 66.31; H, 4.61; N, 15.83.

*5.1.6.7. Ethyl 3-benzyl-9-butyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylate (**10f**).* Method (**vi**): yield 76%, mp 142–144 °C. ^{1}H NMR ($CDCl_3$): δ 8.58 (d, 1H, *J* = 8.8 Hz, H-5), 8.52 (s, 1H, H-8), 7.45–7.30 (m, 6H, H-4 + 5 phenyl-H), 5.89 (s, 2H, C*H*$_2$Ph), 5.08 (d, 2H, *J* = 7.2 Hz, N–C*H*$_2$–CH$_2$–CH$_2$–CH$_3$), 4.41 (q, 2H, *J* = 7.0 Hz, O–C*H*$_2$–CH$_3$), 1.98 (m, 2H, N–CH$_2$–C*H*$_2$–CH$_2$–CH$_3$), 1.68–1.24 (m, 8H, N–CH$_2$–CH$_2$–C*H*$_2$–C*H*$_3$ + OCH$_2$C*H*$_3$). MS *m*/*z* 404 (M^+). Anal. Calcd for $C_{23}H_{24}N_4O_3$: C, 68.30; H, 5.98; N, 13.85. Found C, 67.94; H, 6.17; N, 14.09.

*5.1.6.8. 3-Benzyl-9-butyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylic acid (**11f**).* Method (**vi**): yield 17%, method (**vii**): yield 96%, mp 235–237 °C. ^{1}H NMR ($CDCl_3$): δ 15.13 (s, 1H, COOH), 9.14 (s, 1H, H-8), 8.44 (d, 1H, *J* = 8.8 Hz, H-5), 7.37 (m, 6H, H-4 + 5 phenyl-H), 6.14 (s, 2H, C*H*$_2$Ph), 5.25 (d, 2H, *J* = 7.2 Hz, N–C*H*$_2$–CH$_2$–CH$_2$–CH$_3$), 1.90 (m, 2H, N–CH$_2$–C*H*$_2$–CH$_2$–CH$_3$), 1.55–1.26 (m, 5H, N–CH$_2$–CH$_2$–C*H*$_2$–C*H*$_3$). MS *m*/*z* 376 (M^+). Anal. Calcd for $C_{21}H_{20}N_4O_3$: C, 67.01; H, 5.36; N, 14.88. Found C, 67.34; H, 5.30; N, 115.15.

*5.1.6.9. Ethyl 9-tert-butyl-3-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylate (**10g**).* Method (**vi**): yield 9%, mp 191–94 °C. ^{1}H NMR ($CDCl_3$): δ 8.68 (s, 1H, H-8), 8.27 (d, 1H, *J* = 8.8 Hz, H-5), 7.91 (d, 1H, *J* = 9.0 Hz, H-4), 4.29 (s, 3H, N–CH$_3$), 4.25 (q, 2H, *J* = 7.0 Hz, O–C*H*$_2$–CH$_3$), 3.36 (s, 9H, C–(C*H*$_3$)$_3$), 1.33 (t, 3H, *J* = 7.0 Hz, O–CH$_2$–C*H*$_3$). MS *m*/*z* 328 (M^+). Anal. Calcd for $C_{17}H_{20}N_4O_3$: C, 62.18; H, 6.14; N, 17.06. Found C, 62.51; H, 6.03; N, 17.40.

*5.1.6.10. 9-Tert-butyl-3-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylic acid (**11g**).* Method (**vi**): yield 7%, method (**vii**): yield 94%, mp > 300 °C. ^{1}H NMR ($CDCl_3$): δ 14.43 (s, 1H, COOH), δ 8.68 (s, 1H, H-8), 8.29 (d, 1H, *J* = 8.8 Hz, H-5), 7.97 (d, 1H, *J* = 9.0 Hz, H-4), 4.45 (s, 3H, N–CH$_3$), 3.29 (s, 9H, C–(C*H*$_3$)$_3$). MS *m*/*z* 300 (M^+). Anal. Calcd for $C_{15}H_{16}N_4O_3$: C, 59.99; H, 5.37; N, 18.66. Found C, 60.36; H, 5.14; N, 18.98.

*5.1.6.11. Ethyl 3-methyl-9-(2-methylallyl)-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylate (**10h**).* Method (**v**): yield 22%, mp 148 °C. ^{1}H NMR ($CDCl_3$): δ 8.78 (s, 1H, H-8), 8.62 (d, 1H, *J* = 8.7 Hz, H-5), 7.65 (d, 1H, *J* = 8.7 Hz, H-4), 5.80 (s, 2H, N–CH$_2$C(CH$_3$)=C*H*$_2$), 4.90 (s, 2H, N–C*H*$_2$C(CH$_3$)=CH$_2$), 4.42 (s, 3H, N–C*H*$_3$), 4.29 (q, 2H, *J* = 7.1 Hz, O–C*H*$_2$–CH$_3$), 1.93 (s, 3H, N–CH$_2$C(C*H*$_3$)=CH$_2$), 1.37 (t, 3H, *J* = 7.1 Hz, O–CH$_2$–C*H*$_3$). MS *m*/*z* 326 (M^+). Anal. Calcd for $C_{17}H_{18}N_4O_3$: C, 62.57; H, 5.56; N, 17.17. Found C, 62.29; H, 5.71; N, 17.01.

*5.1.6.12. 3-Methyl-9-(2-methylallyl)-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylic acid (**11h**).* Method (**v**): yield 17%, method (**vii**): yield 91%, mp 167 °C. ^{1}H NMR ($CDCl_3$): δ 15.10 (s, 1H, COOH), 8.78 (s, 1H, H-8), 8.62 (d, 1H, *J* = 8.7 Hz, H-5), 7.65 (d, 1H, *J* = 8.7 Hz, H-4), 5.80 (s, 2H, N–CH$_2$C(CH$_3$)=C*H*$_2$), 4.90 (s, 2H, N–C*H*$_2$C(CH$_3$)=CH$_2$), 4.42 (s, 3H, N–C*H*$_3$), 1.93 (s, 3H, N–CH$_2$C(C*H*$_3$)=CH$_2$). MS *m*/*z* 298 (M^+). Anal. Calcd for $C_{15}H_{14}N_4O_3$: C, 60.40; H, 4.73; N, 18.78. Found C, 60.11; H, 4.95; N, 18.49.

*5.1.6.13. Ethyl 9-(cyclopropylmethyl)-3-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylate (**10i**).* Method (**v**), yield 19%, mp 183–85 °C. ^{1}H NMR ($CDCl_3$): δ 9.08 (s, 1H, H-8), 8.53 (d, 1H, *J* = 9.2 Hz, H-5), 7.89 (d, 1H, *J* = 9.2 Hz, H-4), 5.20 (d, 2H, *J* = 7.8 Hz, N–C*H*$_2$–CH–(CH$_2$)$_2$), 4.47 (s, 3H, N–C*H*$_3$), 4.34 (q, 2H, *J* = 7.2 Hz, O–C*H*$_2$–CH$_3$), 1.57 (m, 1H, N–CH$_2$–C*H*–(CH$_2$)$_2$), 1.31 (t, 3H, *J* = 7.2 Hz, O–CH$_2$–C*H*$_3$), 0.65–0.62 (m, 4H, N–CH$_2$–CH–(C*H*$_2$)$_2$). MS *m*/*z* 326 (M^+). Anal. Calcd for $C_{17}H_{18}N_4O_3$: C, 62.57; H, 5.56; N, 17.17. Found C, 62.88; H, 5.41; N, 17.40.

*5.1.6.14. 9-(Cyclopropylmethyl)-3-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylic acid (**11i**).* Method (**v**), yield 14%, method (**vii**): yield 97%, mp 293–95 °C. ^{1}H NMR (DMSO + $CDCl_3$): δ 15.41 (s, 1H, COOH), 9.08 (s, 1H, H-8), 8.53 (d, 1H, *J* = 9.2 Hz, H-5), 7.89 (d, 1H, *J* = 9.2 Hz, H-4), 5.20 (d, 2H, *J* = 7.8 Hz, N–C*H*$_2$–CH–(CH$_2$)$_2$), 4.47 (s, 3H, N–C*H*$_3$), 2.62 (m, 1H, N–CH$_2$–C*H*–(CH$_2$)$_2$), 0.65–0.62 (m, 4H, N–CH$_2$–C*H*–(C*H*$_2$)$_2$). MS *m*/*z* 298 (M^+). Anal. Calcd for $C_{15}H_{14}N_4O_3$: C, 60.40; H, 4.73; N, 18.78. Found C, 60.11; H, 4.95; N, 18.49.

*5.1.6.15. Ethyl 9-(cyclobutylmethyl)-3-methyl-6-oxo-6,9-dihydro-3H-[1–3]triazolo[4,5-h]quinoline-7-carboxylate (**10j**).* Method (**v**), yield 31%, mp 179–81 °C. ^{1}H NMR ($CDCl_3$): δ 9.15 (s, 1H, H-8), 8.47 (d, 1H, *J* = 8.4 Hz, H-5), 8.08 (d, 1H, *J* = 8.4 Hz, H-4), 5.36 (d, 2H, N–C*H*$_2$–CH–(CH$_2$)$_3$), 4.46 (s, 3H, N–C*H*$_3$), 4.19 (q, 2H, *J* = 7.0 Hz, O–C*H*$_2$–CH$_3$), 2.89 (m, 1H, N–CH$_2$–C*H*–(CH$_2$)$_3$), 2.06–1.89 (m, 6H, N–CH$_2$–CH–(CH$_2$)$_3$), 1.37 (t, 3H, *J* = 7.0 Hz, O–CH$_2$–C*H*$_3$). MS *m*/*z* 340 (M^+). Anal. Calcd for $C_{18}H_{20}N_4O_3$: C, 63.52; H, 5.92; N, 16.46. Found C, 63.85; H, 6.12; N, 16.11.

*5.1.6.16. 9-(Cyclobutylmethyl)-3-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylic acid (**11j**).* Method (**v**), yield 12%, method (**vii**): yield 95%, mp 222–228 °C (dec). ^{1}H NMR (DMSO): δ 15.50 (s, 1H, COOH), 9.15 (s, 1H, H-8), 8.47 (d, 1H, *J* = 8.4 Hz, H-5), 8.08 (d, 1H, *J* = 8.4 Hz, H-4), 5.36 (d, 2H, N–C*H*$_2$–CH–(CH$_2$)$_3$), 4.46 (s, 3H, N–C*H*$_3$), 2.89 (m, 1H, N–CH$_2$–C*H*–(CH$_2$)$_3$), 2.06–1.89 (m, 6H, N–CH$_2$–CH–(C*H*$_2$)$_3$). MS *m*/*z* 312 (M^+). Anal. Calcd for $C_{16}H_{16}N_4O_3$: C, 61.53; H, 5.16; N, 17.94. Found C, 61.08; H, 5.01; N, 17.56.

5.2. Microbiology

5.2.1. Anti-mycobacterial assays

Anti-mycobacterial activity was determined by the twofold agar dilution method against *M. tuberculosis* H37*Rv* (ATCC 27294), H37*Ra*, *M. smegmatis* mc^{2}155 and *M. bovis* BCG. Strains were grown in Middlebrook 7H11 medium supplemented with OADC. The bacterial suspensions (at the standard turbidity of 1 MACFARLAND) were diluted 1:10, 1:100 and 1:10,000 with Middlebrook 7H9 and inoculated in duplicate Middlebrook 7H11 plates containing serial concentrations of the test compounds. Antimycobacterial activity against *M. tuberculosis* H37Ra, *M. smegmatis* mc^{2}155 and *M. bovis* BCG were performed by the broth microdilution methodology according to NCCLS [38]. Stock cultures were prepared from isolated

colonies selected on Middlebrook 7H11 agar plates and diluted in Middlebrook 7H9. Final bacterial inocula were approximately 1–5 × 10^5 CFU/mL. Assays were performed in sterile 96-well microtiter plates with round bottom wells, sealed in plastic bag. Plates were incubated at 37 °C and read after 72 h or 7 days for *M. smegmatis* mc^2155 and *M. bovis* BCG, respectively.

5.2.2. Anticandida assays

MICs against *C. albicans* and *C. tropicalis* were determined according to NCCLS [39]. Fungal suspensions were obtained from cultures incubated at 35 for 48 h in Sabouraud Dextran agar. Colonies were collected and diluted to a density of 10^6 CFU/mL. Test compounds were dissolved in dimethyl sulfoxide at an initial concentration of 10 mg/mL and serially diluted in RPMI-1640. A 50 μL sample of the above serial dilutions of test compounds were added, in, sterile 96-well microtiter plates with round bottom wells, to equal volume of fungal suspensions and incubated at 35 °C for 48–72 h. MIC determination was performed in duplicate.

5.2.3. Antibacterial assays

All Gram-positive and Gram-negative bacteria were clinical or laboratory strains, prepared from isolated colonies selected on Mueller Hinton Agar (MHA). MIC assays were performed by broth microdilution methodology in sterile 96-well microtiter plates, according to CLSI procedure [40], using final inocula of 1–5 × 10^5 CFU/mL in Mueller Hinton Broth. Test compounds were dissolved in dimethyl sulfoxide at an initial concentration of 10 mg/mL and serially diluted in Mueller Hinton Broth. A 50 μL sample of each drug concentration was distributed in the wells containing the same volume of the bacterial inocula and the plates were incubated at 37 °C for 18–24 h. MIC was determined as the minimum concentration inhibiting visible growth. MIC determination was performed in duplicate.

5.2.4. Cytotoxicity assays

Exponentially growing cells derived from human hematological tumors [CD4+ human T-cells containing an integrated HTLV-1 genome (MT-4)] were seeded at an initial density of 1 10^5 cells/mL in 96-well plates in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 U/mL penicillin G, and 100 g/mL streptomycin. Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. 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 [41].

Acknowledgement

The Fondazione Banco di Sardegna is gratefully acknowledged for financial support.

References

[1] A.R. Katritzky, Farmaco, Ed. Sci. 43 (1988) 1175–1181.
[2] A.R. Katritzky, S. Rachwal, G.J. Hitchings, Tetrahedron 47 (1991) 2683–2732.
[3] A.R. Katritzky, J. Jiang, J.V. Greenhill, P.J. Steel, J. Chem. Soc. Perkin Trans. 1 (1992) 3055–3060.
[4] F. Sparatore, F. Pagani, Farmaco, Ed. Sci. 17 (1962) 414–429.
[5] F. Sparatore, F. Pagani, Farmaco, Ed. Sci. 20 (1965) 248–258.
[6] F. Sparatore, M.I. La Rotonda, G. Paglietti, E. Ramundo, C. Silipo, A. Vittoria, Farmaco, Ed. Sci. 33 (1978) 901–923.
[7] F. Sparatore, F. Pagani, G. Caliendo, E. Novellino, C. Silipo, A. Vittoria, Farmaco, Ed. Sci. 43 (1988) 141–151.
[8] A. Boido, I. Vazzana, F. Sparatore, B. Cappello, V. Diurno, C. Matera, R. Marrazzo, S. Russo, M.L. Cenicola, E. Marmo, Farmaco 44 (1989) 257–277.
[9] M. Loriga, G. Paglietti, F. Sparatore, G. Pinna, A. Sisini, Farmaco 47 (1992) 439–448.
[10] G. Paglietti, P. Sanna, A. Carta, F. Sparatore, I. Vazzana, A. Peana, M. Satta, Farmaco 49 (1994) 693–702.
[11] I. Vazzana, A. Boido, F. Sparatore, R. Di Carlo, G.M. Raso, M. Pacilio, Farmaco 52 (1997) 131–139.
[12] G. Paglietti, F. Sparatore, Farmaco, Ed. Sci. 27 (1972) 380–396.
[13] G. Paglietti, F. Sparatore, Ann. Chim. 62 (1972) 128–143.
[14] A. Carta, P. Sanna, G. Paglietti, F. Sparatore, Heterocycles 55 (2001) 1133–1140.
[15] P. Sanna, A. Carta, G. Paglietti, S. Zanetti, G. Fadda, Farmaco 47 (1992) 1001–1019.
[16] P. Sanna, A. Carta, G. Paglietti, A. Bacchi, G. Pelizzi, J. Heterocycl. Chem. 34 (1997) 97–106.
[17] A. Carta, P. Sanna, H. Molinari, Heterocycles 45 (1997) 1391–1395.
[18] P. Sanna, A. Carta, G. Paglietti, Heterocycles 51 (1999) 2171–2181.
[19] A. Carta, P. Sanna, A. Bacchi, Heterocycles 57 (2002) 1079–1090.
[20] P. Sanna, A. Carta, M.E. Rahbar Nikookar, Eur. J. Med. Chem. 35 (2000) 535–543.
[21] P. Sanna, A. Carta, L. Gherardini, M.E. Rahbar Nikookar, Farmaco 57 (2002) 79–87.
[22] A. Carta, P. Sanna, M. Palomba, L. Vargiu, M. La Colla, R. Loddo, Eur. J. Med. Chem. 37 (2002) 891–900.
[23] A. Carta, M. Palomba, G. Boatto, B. Busonera, M. Mureddu, R. Loddo, Farmaco 59 (2004) 637–644.
[24] K. Fries, H. Guterbock, C.H. Kuhn, Liebigs Ann. Chem. 511 (1934) 213.
[25] R.H. Slater, J. Chem. Soc. (1932) 2196.
[26] M.G. Ferlin, I. Castagliuolo, G. Chiarelotto, J. Heterocycl. Chem. 39 (2002) 631–638.
[27] A. Nuvole, P. Sanna, G. Paglietti, C. Juliano, P. Cappucinelli, Il Farmaco 44 (1989) 619–632.
[28] P. Sanna, A. Carta, G. Paglietti, S. Zanetti, G. Fadda, Il Farmaco 47 (1992) 1001–1019.
[29] P. Sanna, P.A. Sequi, G. Paglietti, Il Farmaco 50 (1995) 47–54.
[30] P. Sanna, A. Carta, G. Paglietti, Heterocycles 53 (2000) 423–432.
[31] A. Carta, G. Boatto, G. Paglietti, G. Poni, M.G. Setzu, P. Caredda, Heterocycles 60 (2003) 833–842.
[32] A. Carta, G. Paglietti, Arkivoc 5 (2004) 66–75.
[33] A. Carta, G. Paglietti, Triazolo[4,5-*g*]quinolines, imidazo[4,5-*g*]quinolines, pyrido[2,3-*g*]quinoxalines and [4,7]phenantrolines. Easy pathway for the syntheses of these *N*-Tricycles. in: T.S. Kaufman, E.L. Larghi (Eds.), Modern Approaches to the Synthesis of O- and N-Heterocycles, vol. 1. Research Signpost, Kerala, India, 2007, pp. 173–185 (Chapter 7).
[34] A. Carta, S. Pricl, S. Piras, M. Fermeglia, P. La Colla, R. Loddo, Eur. J. Med. Chem. 44 (2009) 5117–5122.
[35] A. Carta, M. Palomba, G. Paglietti, P. Molicotti, B. Paglietti, S. Cannas, S. Zanetti, Bioorg. Med. Chem. Lett. 17 (2007) 4791–4794.
[36] A. Carta, S. Piras, M. Palomba, P. Molicotti, S. Zanetti, Anti-Infect. Agents Med. Chem. 7 (2008) 134–147.
[37] R. E. Diehl, R. V. Kendall, US patent 4,086,242 (25 April 1978), Chem. Abstr. 89 (1978) 109512g.
[38] National Committee for Clinical Laboratory Standards: Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard 2006 – NCCLS document M24–A. NCCLS, Wayne, PA, 2006.
[39] National Committee for Clinical Laboratory Standards: Reference Methods for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard – Second Edition – 2002 – NCCLS document M27–A2. NCCLS, Wayne, PA, 2006.
[40] National Committee for Clinical Laboratory Standards: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard – Seventh Edition – 2006 – NCCLS document M7–A7. NCCLS, Wayne, PA, 2006.
[41] R. Pauwels, J. Balzarini, M. Baba, R. Snoeck, D. Schols, P. Herdewijn, J. Desmyster, E. De Clercq, J. Virol. Methods 20 (1998) 309–321.