Design, synthesis and antitubercular activity of 4-alkoxy-triazoloquinolones able to inhibit the *M. tuberculosis* DNA gyrase

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ABSTRACT

A number of new F-triazolequinolones (FTQs) and alkoxy-triazolequinolones (ATQs) were designed, synthesized and evaluated for their activity against *Mycobacterium tuberculosis* H37Rv. Five out of 21 compounds exhibited interesting minimum inhibitory concentration (MIC) values (6.6–57.9 μM), ATQs generally being more potent than FTQs. Two ATQs, **21a** and **30a**, were endowed with the best anti-Mtb potency (MIC = 6.9 and 6.6 μM, respectively), and were not cytotoxic in a Vero cell line. Tested for activity against *M. tuberculosis* DNA gyrase in a DNA supercoiling activity assay, **21a** and **30a** showed IC_{50} values (27–28 μM) comparable to that of ciprofloxacin (10.6 μM). **21a** was next selected for screening against several Mtb strains obtained from clinical isolates, including multi-drug-resistant (MDR) variants. Importantly, this compound was effective in all cases, with very promising MIC values (4 μM) in the case of some isoniazid/rifampicin-resistant Mtb strains. Finally, computer-based simulations revealed that the binding mode of **21a** in the Mtb gyrase cleavage core complexed with DNA and the relevant network of intermolecular interactions are utterly similar to those described for ciprofloxacin, yielding a molecular rationale for the comparable anti-mycobacterial and DNA gyrase inhibition activity of this quinolone.

1. Introduction

Mycobacterium tuberculosis (Mtb), the etiological agent of tuberculosis (TB), is one of the most important pathogens of humans, second only to the HIV in the number of deaths caused annually [1]. Mtb is estimated to latently infect one-third of the world's population (≈2 billion people), thereby creating a huge reservoir for future disease. In 2016 only, 10.4 million of the infected people developed active TB and the death toll from this disease reached 1.7 million. At the same time, 6.3 million new cases of TB were reported (up from 6.1 million in 2015), equivalent to 61% of the estimated incidence of 10.4 million [1]. Control of Mtb is hindered by multidrug-resistant (MDR)-TB, characterized by the presence of Mtb strains resistant to two primary anti-TB drugs, rifampicin and isoniazid. Drug-resistant TB is a persistent threat, with 490000 cases of MDR-TB emerging in 2016 and additional 110000 cases that were susceptible to isoniazid but resistant to rifampicin (RR-TB), the most effective first-line anti-TB drug. Even more frightening is the emergence of extensively drug resistant TB (XDR-TB) reported in all regions of the world. XDR-TB involves resistance to isoniazid and rifampicin in addition to resistance to any fluoroquinolone and one injectable second-line drug. The reasons why MDR-TB continues to emerge and spread are mismanagement of TB treatment and person-to-person transmission [2]. Inappropriate or incorrect use of antimicrobial drugs, use of ineffective drug formulations, and premature treatment interruption can all lead to resistant form of TB, which can be further transmitted, particularly in crowded or at-risk settings such as prisons and hospitals.

At present, Europe is facing TB recrudescence due to the human migration flux from TB high-incidence countries [3]. This is a relevant phenomenon, increasing over time, considering that a significant proportion (up to 70%) of TB cases notified in Europe are

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associated to foreign-born populations. Irregular immigration represent one of the main problems, since the migratory journey, often occurring under precarious conditions, increases the TB risk of migrants [4].

Under this glooming perspective, the discovery and development of new anti-TB drugs is very pressing and challenging at the same. Indeed, to be highly effective any novel Mtb inhibitor should be ideally endowed with acceptable tolerability profile (particularly in HIV-TB co-infected patients), high efficacy to reduce treatment duration, be active against MDR/XDR and latent TB forms.

Basically, the road to new anti-Mtb agents can be paved in different ways. One of these approaches consists in the exploration of known and clinically validated bacterial targets using new chemical entities or the modification of existing drug classes ultimately limiting/avoiding cross resistance phenomena. Fluoroquinolones constitute a class of synthetic antibiotics that inhibit type-II topoisomerases (i.e., DNA gyrase and topoisomerase IV) in most bacterial species [5–8]. DNA gyrase is an ATP-dependent heterotetrameric ($GyrA_2GyrB_2$) enzyme that acts by creating a transient double-stranded DNA break. It is unique in catalyzing the negative supercoiling of DNA and is essential for efficient DNA replication, transcription, and recombination. Blocking the activity of this protein thus results in pathogen cell death. Also, since DNA gyrase is the sole type II topoisomerase in Mtb, this enzyme is likely the only target for fluoroquinolones in this microorganism [9,10]. Accordingly, a novel inhibitor of Mtb DNA gyrase would be effective against MDR-Mtb, could be active against fluoroquinolone-resistant Mtb strains, and could be active against non-replicating mycobacteria, another important aspect in the eradication of persistent infections [11].

In the past several molecules active against Mtb were proposed by our group [12–15]. More recently, [1,2,3]triazolo[4,5-h]quinolone (TQ) derivatives were also identified by us as new chemical entities able to inhibit replication of MDR/XDR-Mtb strains [16–19]. Some of these compounds (Fig. 1) were highly potent against H37Rv, H37Ra and further 11 clinical isolates of MDR/XDR-TB strains; concomitantly, they were devoid of cytotoxicity.

With the aim of further enhancing the biological activity of the [1,2,3]triazolo[4,5-h]quinolone scaffold, in the present effort we designed and synthesized a number of new F-triazolequinolones (FTQs) and alkoxy-triazolequinolones (ATQs). Testing all TQ derivatives against Mtb H37Rv highlighted **21a** and **30a** as the most active compounds in the full set. *In vitro*, we found that **21a** and **30a** were both effective at inhibiting DNA supercoiling by Mtb gyrase, with IC_{50} values comparable to that of ciprofloxacin (CPF), a quinolone-derivative antibiotic used in TB therapy. Further testing carried out with **21a** confirmed that this compound was quite active against a panel of several other Mtb strains, including a set of multi-drug-resistant variants obtained from clinical isolated. Finally, *in silico* modeling revealed that binding mode of **21a** in the Mtb gyrase cleavage core complexed with DNA and the relevant network of intermolecular interactions were utterly similar to those described for ciprofloxacin, yielding a molecular rationale for the comparable antibacterial and DNA gyrase inhibition activity of these two quinolones.

2. Chemistry

SAR considerations on previously synthesized TQs revealed that the most active derivatives featured a small and lipophilic alkyl pendant on the triazole moiety. In particular, the presence of a methyl group on the triazole N-3 atom was found to enhance the antitubercular activity of the TQ, while the same substituent on the N-1 counterpart drastically reduced it [6,7,9]. Starting from this evidence, in this work we reasoned that, preserving the favorable $-CH_3$ group on the triazole moiety and introducing new substituents at different positions of the TQ scaffold we could obtain a new series of efficient Mtb inhibitors (Fig. 2).

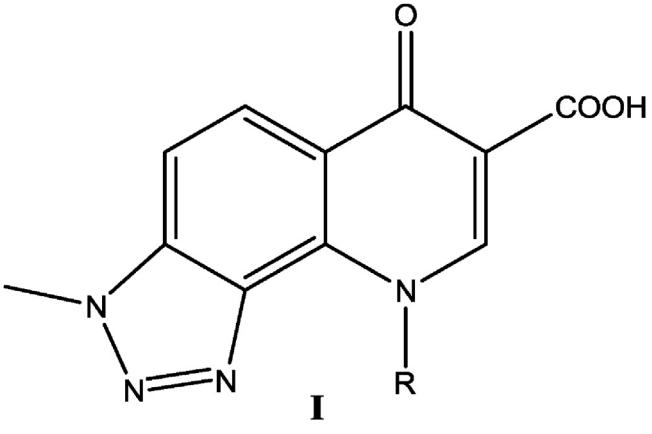
Accordingly, we prepared two compounds bearing different lipophilic alkyl groups on the quinolone N-9 atom (R_1 = methyl and ethyl). Since fluoroquinolones are included in standard regimens for the treatment of a variety of community and nosocomial infections, we also explored the effect of the presence of a fluorine at position 4 of the TQ scaffold (R_2). All new compounds were prepared both in their free acid and ethyl ester form.

The synthesis of the intermediate 7-fluoro-1*H*-benzo[*d*][1,2,3]triazole (**6**) was already reported by Kirk et al., in 1969 [20]; however, the process required a substantial adaptation to obtain the 2-fluoro-6-nitroaniline (**4**) in good yield, as shown in Scheme 1.

Methylation of **6** was carried out using dimethyl sulfate in NaOH 2 M, as reported in Scheme 2.

The reaction yielded a mixture of three isomers (7-fluoro-1-methyl-1*H*-benzotriazole (**7a**), 4-fluoro-2-methyl-2*H*-benzotriazole (**7b**), and 4-fluoro-1-methyl-1*H*-benzotriazole (**7c**)) which were separated by chromatography and identified by NMR [21]. Starting from compound **7a**, the synthesis of the intermediate ethyl-4-fluoro-3-methyl-6-oxo-6,9-dihydro-3*H*-[1,2,3] triazole [4,5-h] quinolone (**11**) was performed as described in Scheme 3.

Alkylation of **11** at N-9 position with the CH_3-I or CH_3-CH_2-I in the presence of potassium hydroxide, followed by hydrolysis in NaOH 2 M, gave the corresponding alkyl derivatives **11** and **15**, respectively (Scheme 4).



I

Fig. 1. 9-Substituted-3-methyl-6-oxo-6,9-dihydro-3*H*-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**I**).

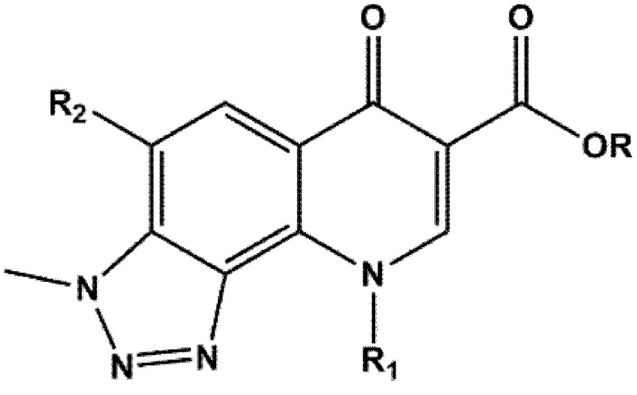
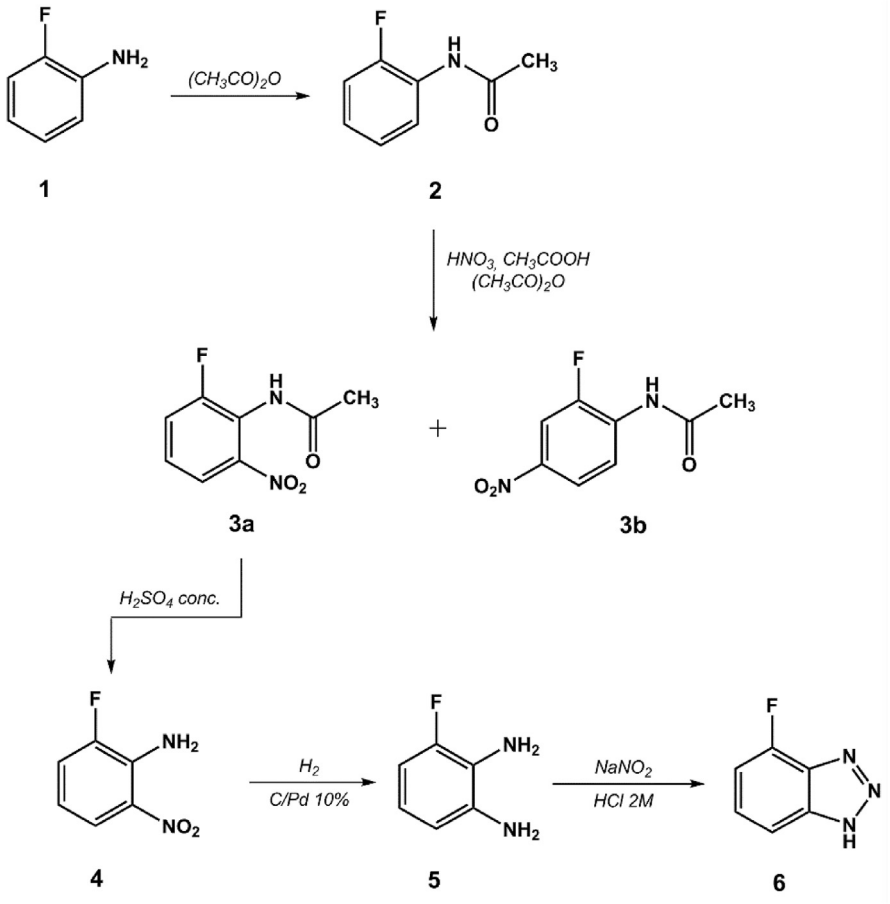
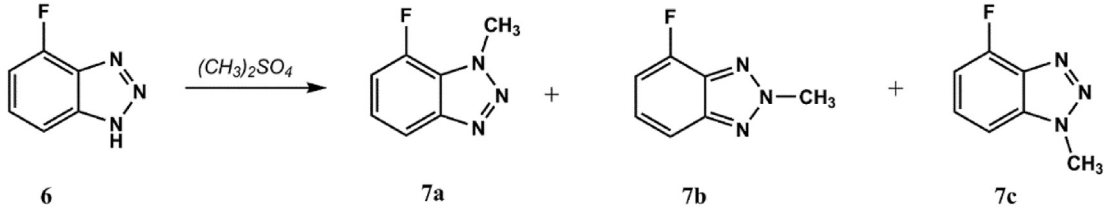


Fig. 2. 4,9-Substituted-3-methyl-6-oxo-6,9-dihydro-3*H*-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**II**).



Scheme 1. Synthesis of the key intermediate 4-fluoro-1*H*-benzotriazole (**6**).



Scheme 2. Synthesis of the 4(7)-fluoro-1(2)-methyl-1*H*-benzotriazole (**7a-c**).

Nucleophilic aromatic substitution of the electron-withdrawing F atom in compounds **7a-c** with alkoxy groups such as $-OCH_3$ and $-OCH_2CH_3$ led us to obtain the new derivatives **21a,c**, **23a-c**, **30a** and **32a** *via* the chemical route depicted in Scheme 5. From the intermediate compounds **19a-c** only one (**21a**) of the three expected TQs was obtained. Indeed, the alkylation of **19c** took place on the oxygen atom at position 4, ultimately leading to the formation of the quinolinic derivatives **20c** and **22c**. Non-alkylated derivatives on N-9 (**24a,c**) were also prepared to evaluate whether the introduction of a methoxy group at position 4 still required the presence of an alkyl at the N-9 position for activity.

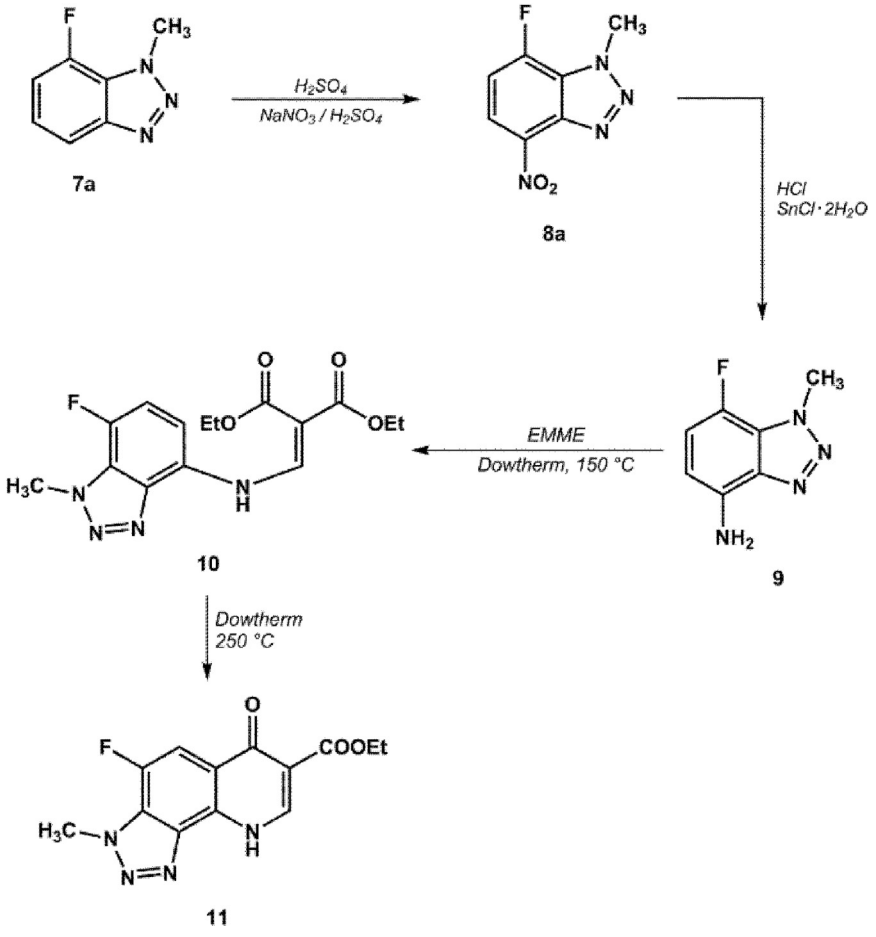
Of note, in the case of 4-ethoxy derivatives only compounds **30a** and **32a** were synthesized because the corresponding derivative, without the methoxy group, of the series "b" and "c" resulted inactive [16].

Finally, two other, unconventional TQs resulted from the reaction of the halogenated benzotriazoles **8a** and **8c** with strong nucleophiles such as ethanethiol or ammonia, respectively. Starting from compound **8a**, the final quinolone **37** was obtained via the 7-ethylthio intermediate derivative **33**, as shown in Scheme 6. On the contrary, starting from **8c** the final quinolone **41** was synthesized according to the pathway shown in Scheme 7. Based on what we saw for the previous series, also in this case we prepared only quinolone derivatives with the methyl on N-3 [16]. Following the same lead, we performed only a methylation on the quinolone moiety, since ethyl derivatives resulted less potent than methylated ones.

3. Results and discussion

3.1. Minimum inhibitory concentration (MIC) determination of the new triazoquinolones against Mtb H37Rv strain

All newly synthesized triazoloquinolones were initially tested against MTb H37Rv in a conventional Resazurin Microtiter Assay (REMA) [22]. The results gathered in Table 1 show that compounds



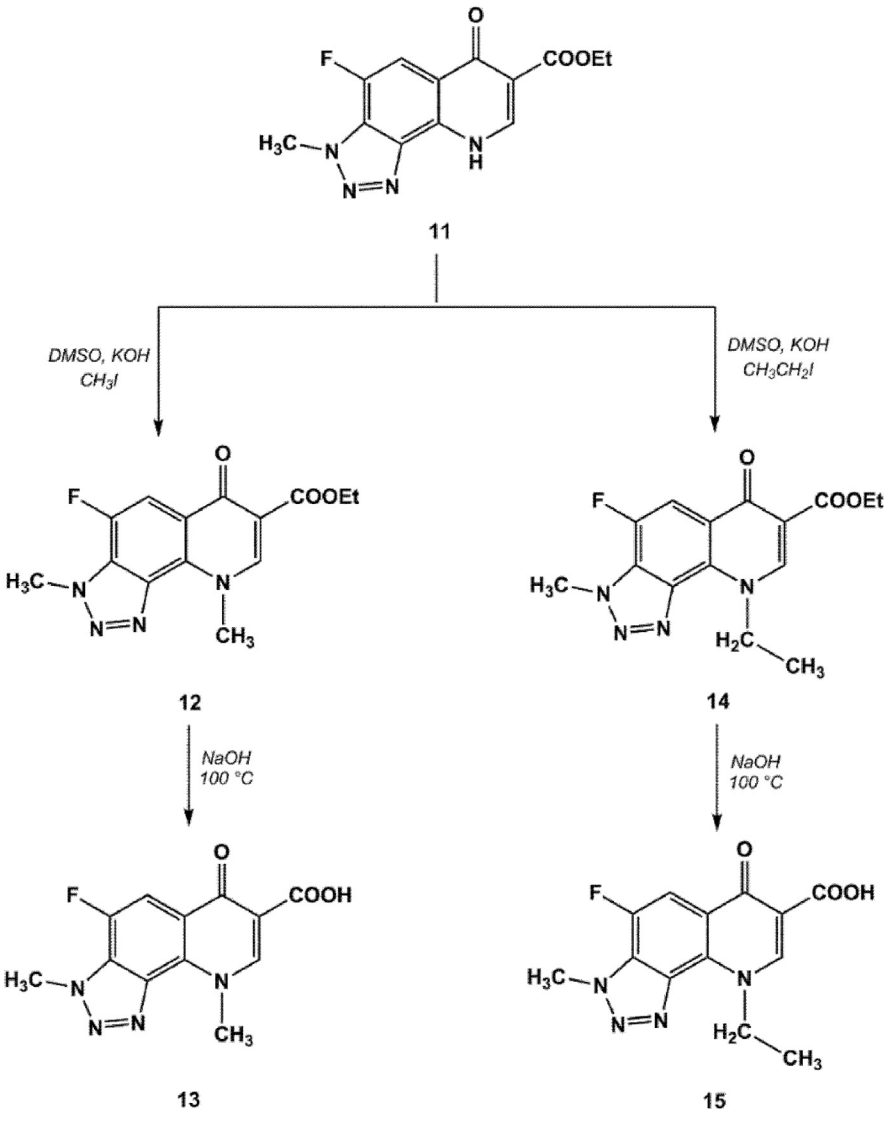
Scheme 3. Synthesis of the key intermediate 4-fluoro-3-methyl-6-oxo-6,9-dihydro-3*H*-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**11**).

13, **20b**, **21a**, **23a**, **30a**, **32a**, and **37** exhibits interesting minimum inhibitory concentration (MIC) values in the range 6.6–57.9 μM. Taking **21a** as a reference compound (MIC = 6.9 μM), the biological data reveal that both the nature and position of the substituents exert an effect on the antimycobacterial activity of the corresponding derivatives. Thus, while the replacement of a methyl group with a longer (-CH_2-CH_3) chain on the O-4 atom of the quinolone moiety does not result in an appreciable change of the corresponding MIC (**30a**, MIC = 6.6 μM), the analogous substitution on the N-9 atom of the quinolone leads to a substantial decrease in activity (**23a**, MIC = 52.9 μM). Finally, the double replacement of an ethyl group at both positions of the quinolone moiety results in an intermediate MIC value for the corresponding compound (**32a**, MIC = 25.3 μM). If a thio-ether is used as in compound (**37**), the antimycobacterial activity is seen to drop (50.3 μM), as it is the case of compounds (**41**) and (**13**), featuring two electron withdrawing groups at the same position (-NO_2, MIC > 100 μM, and −F, MIC = 57.9 μM, respectively). The position of the methyl substituent on the triazole ring is also found to have an effect on the activity of these series of compounds. Indeed, with respect to (**21a**) the quinolone (**20b**) and the quinoiline (**21c**), in which the −CH_3 group is located on the N atom at position 2 or 1 of the triazole ring, respectively, show MIC values of 27.8 μM and >100 μM, respectively. Finally, compounds (**20a** and **24a**) were synthesized and tested to verify the fundamental role played by the −COOH substitution at positions 7 (**20a**) and the −CH_3 and on the N-9 (**24a**) of the quinolone ring in the activity of these molecules. As expected, both compounds were devoid of antimycobacterial activity (MIC > 100 μM).

All compounds were also examined for in vitro cytotoxicity in the mammalian VERO cell line by the MTT colorimetric assay. The results, expressed as % cell viability in the presence of at 100 μM of each compound, are summarized in the last column of Table 1. Cell viabilities ranged from 81.2 to 94.3%, confirming that all molecules did not exhibit any significant toxicity effect on the selected cell line.

3.2. *In silico* prediction of pharmacokinetic parameters for compounds ***13***, ***20b, 21a, 23a, 24a, 30a, 32a***, *and* ***37***

All compounds endowed with activity against Mtb H37Rv (compounds **13**, **20b**, **21a, 23a**, **30a, 32a**, and **37**, Table 1) and **24a,** as negative control, were subjected to *in silico* prediction of a selection of pharmacokinetic parameters, i.e., molecular weight (MW), numbers of hydrogen bond donors/acceptors (HBD/HBA), water-octanol partition coefficient (logP), water solubility (logS) and polar surface area (PSA)(Table 2). For comparison purposes, ciprofloxacin was also analyzed as the reference standard. All molecular properties were analyzed on the basis of the extended version of the Lipinski rule of five [23,24], according to which an orally active drug should not violate more than one of the following criteria: i) MW ≤ 500; ii) HBD and HBA (linked to compound membrane permeability) ≤ 5 and ≤ 10, respectively; iii) logP and logS (helpful in predicting the intestinal absorption and consequent efficiency of the trans-cellular transport of the drug) ≤ 5; iv) PSA < 140 $Å^2$. As seen from Table 3, all compounds exhibited good drug likeness



Scheme 4. Synthesis of 4-fluoro-3,9-dimethyl-6-oxo-6,9-dihydro-3*H*-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**13**) and 4-fluoro-3-methyl-9-ethyl-6-oxo-6,9-dihydro-3*H*-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**15**).

properties and did not violated any of the pharmacokinetic parameters as proposed by Lipinski's rule, suggesting these molecules possess properties that would make them likely orally active drugs in humans.

3.3. MIC determination of ***21a*** against clinical isolates of *Mycobacterium tuberculosis*

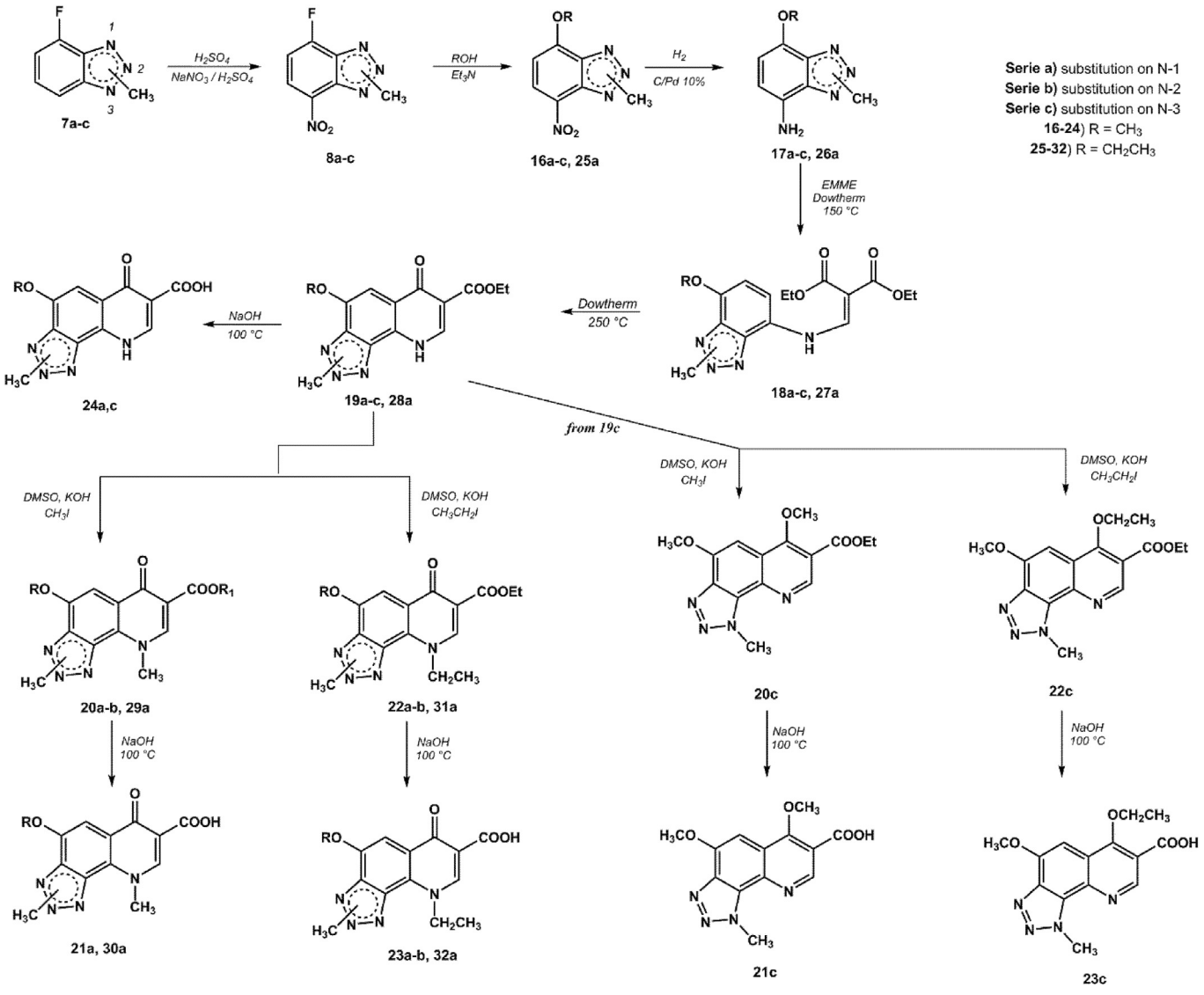
Compounds **21a** was also tested against 21 different Mtb strains obtained from clinical isolates. Among these, 4 multi-drug-resistant (MDR) and 3 ciprofloxacin-resistant strains were included. Mtb strains were defined as those that did not respond to least both isoniazid and rifampicin, the two anti-Mtb drugs currently adopted in the clinics. Table 3 reports these results, together with the MIC values for ciprofloxacin against the identified resistant. MIC values of **21a** and ciprofloxacin against Mtb strain H37Rv are also shown for comparison.

While compound **21a** was active on all identified Mtb resistant strains with MIC values in the range 4–32 μM), it is of particular interest to observe that this TQ derivative is remarkably active on 3 MDR strains, i.e., strains 7 and 1120 (MIC = 4 μM), and strain 512 (MIC = 8 μM).

3.4. Inhibition of Mtb DNA gyrase supercoiling activity by ***21a*** and ***30a***

The two most active compounds **21a** and **30a** (MIC = 6.9 and 6.6 μM, Table 1) were next assessed for their ability to inhibit Mtb DNA gyrase in a DNA supercoiling activity assay. A less active compound **24a** was also selected as negative control while ciprofloxacin was used as positive control. When compared to ciprofloxacin ($IC_{50} = 10.6 \pm 0.60$ μM), compounds **21a** and **30a** were found to be equally active, with IC_{50} values of 27.6 ± 0.71 and 28.2 ± 1.56 μM, respectively (Fig. 3). As expected, compound **24a** did not show comparable inhibition of the enzyme ($IC_{50} = 91.3 \pm 1.56$ μM).

Finally, in order to increase the knowledge of this new class of quinolones, derivatives **21a** and **30a** were assesses for activity against a large panel of different microorganisms, including Gram positive and Gram-negative bacteria (*S. aureus*, *S. typhimurium*, *E. faecalis* and *E. coli*) and yeasts (*C. albicans* and *C. tropicalis*). Pleasingly, both compounds resulted devoid of antimicrobial



Scheme 5. Synthesis of 4-alkoxy-triazoloquinolones (**21a,c 23a,b,c, 30a** and **32a**).

activity (MIC> 100 μM).

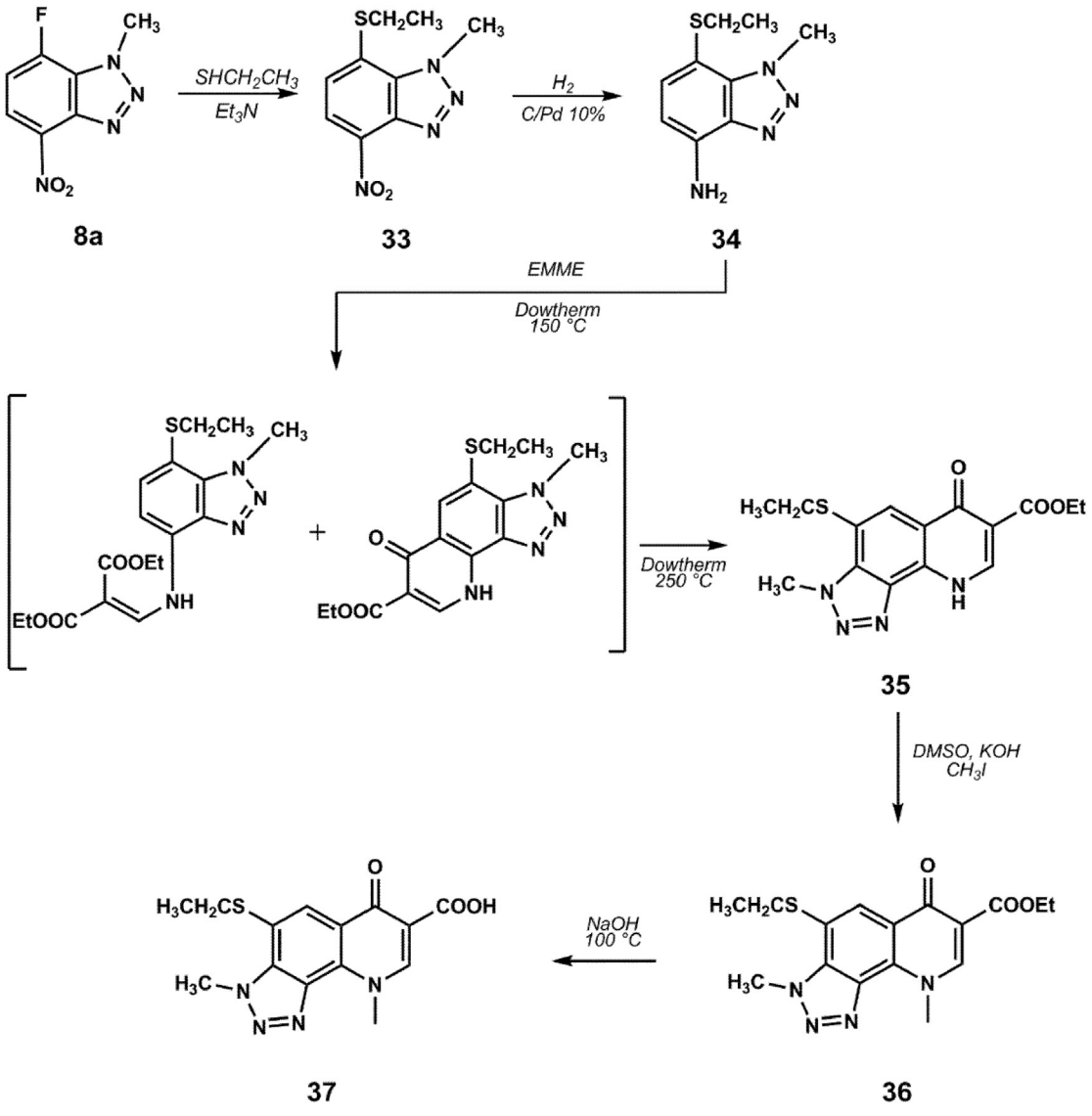
3.5. Molecular modeling study of the binding of compound *21a* to the MTb DNA gyrase

The putative interactions of the most active compound **21a** with the Mtb DNA gyrase were next investigated by molecular dynamics (MD) simulations [25–27]. The available crystallographic structure of the *Mtb DNA gyrase* (pdb code 5BTC) in complex with ciprofloxacin (**CPF**) [28] was used as the starting point for these computational studies. As shown in Fig. 4A, the predicted binding mode of **21a** in the Mtb DNA gyrase active site is very similar to that characterizing ciprofloxacin and other related quinolones. Indeed, the keto-acid group of **21a** coordinates a magnesium ion while the triazole ring performs weak van der Waals interactions with the neighboring GyrB residues Arg482, Thr500, and Glu501. In addition, other favorable contacts are detected between **21a** structure GyrA residues Asp94, Arg128, and Ptr129. These favorable interaction network translates in a good affinity of **21a**, the corresponding free energy of binding (ΔG_{bind}) value being equal to -6.88 ± 0.19 kcal/mol, in agreement with the experimental micromolar inhibitory activity of the compound.

Fig. 4B compares the conformations adopted by ciprofloxacin and compound **21a** in the Mtb DNA gyrase as extracted from the corresponding equilibrated MD simulation trajectories. As seen from this Figure, the two molecules can be largely superposed, the slightly more favorable ΔG_{bind} value (-7.29 ± 0.21 kcal/mol) calculated for the **CPF**/gyrase complex being in line with a 4.5-fold higher potency of ciprofloxacin compared to **21a** ($EC_{50} = 1.5$ μM and 6.9 μM respectively, Table 1).

4. Conclusion

In summary in this study we can assert that 4-methoxy (or ethoxy)-3,9-dimethyl-6-oxo-6,9-dihydro-3*H*-[1,2,3]triazolo[4,5-h] quinoline-7-carboxylic acids (**21a** and **30a**) are endowed with antimycobacterial activity. Surprisingly, on the contrary to what happens in the case of fluoroquinolones, the presence of a fluorine in the classic 6 position of fluoroquinolones (corresponding to 4 of the TQs) does not involve an increase in biological activity but a drastic decrease. The same negative effect occurs with the substitution of the alkaloxy group with an ethanethiol or with a nitro group. It is



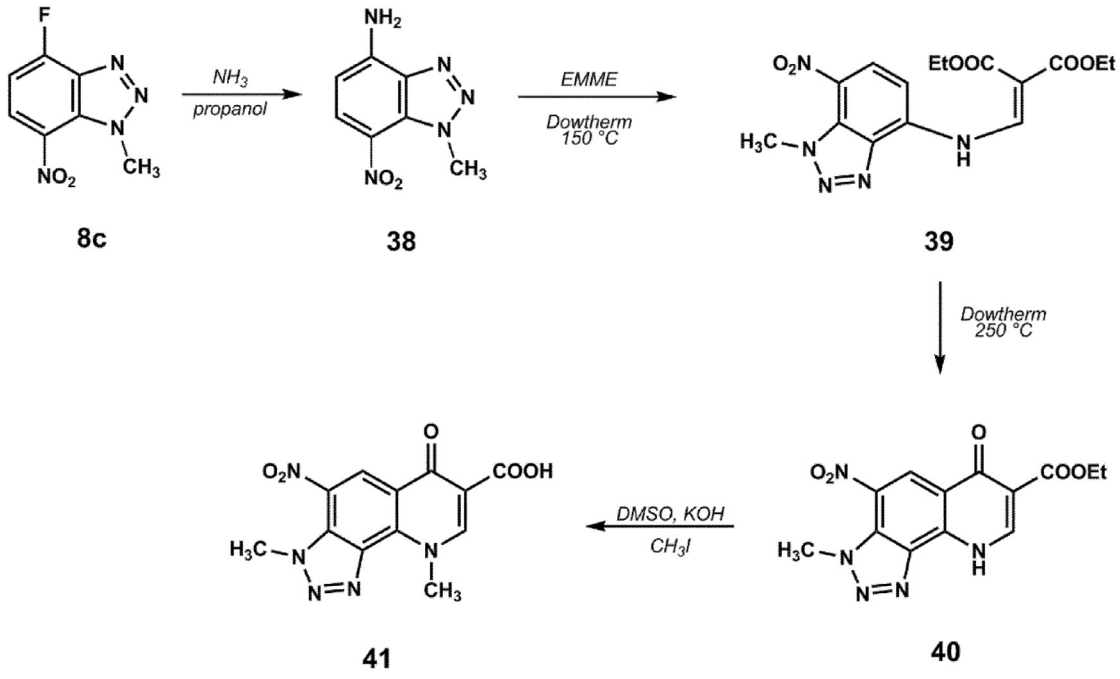
Scheme 6. Synthesis of 4-(ethylthio)-3,9-dimethyl-6-oxo-6,9-dihydro-3*H*-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**37**).

equally surprising that the most active substituent on the N-9 of the TQs (corresponding to the N-1 of the classic fluoroquinolones) is methyl and not ethyl. Moreover, the activity, although not very high, of the compound 4-methoxy-2,9-dimethyl-6-oxo-6,9-dihydro-2H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**20b**) which differs from **21a** by the displacement of methyl from position 3 to 2 of the benzotriazole ring and of an ester group in place of a carboxylic acid, reinforces the hypothesis of the different location of these molecules within the *M. tuberculosis* DNA gyrase. The different mechanism of action of this new class of quinolones is further demonstrated by their complete lack of activity against bacteria. This aspect is very important because it avoids interference with the intestinal bacterial flora and reduces the risk of vertical transmission of resistance across species. Interestingly, an analysis of different bacterial DNA gyrases revealed that these enzymes share high sequence similarity and a highly conserved active site [28]. As such, an hypothesis based on different binding modes of the present compounds on their biological targets can be sensibly discarded as the molecular rationale underlying their selective behavior towards the Mtb DNA gyrase, and further microbiological studies are needed to explain the specificity of action of these new triazolequinolones against Mycobacterium tuberculosis. Moreover, derivative **21a**, chosen as lead compound, has generally shown to maintain the same activity in clinical strains including MDRs. Although its ability to inhibit M. tuberculosis DNA gyrase and its in vitro potency are slightly lower than that of ciprofloxacin, **21a** do not demonstrate any loss of potency against ciprofloxacin resistant strains. Finally, on the basis of pharmacokinetic parameters and the drug solubility with PVP10, supported also by the good cytotoxicity profile, suggest to us to submit the compound **21a** further experiments in vivo.

5. Experimental section

5.1. Chemistry

Melting points were carried out with a Köfler hot stage or Digital Electrothermal melting point apparatus. UV analysis was carried out using a UV–Vis Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were determined in $CDCl_3$ or DMSO-δ_6 and were recorded with a Bruker Avance III 200 and 400 NanoBay.



Scheme 7. Synthesis of 3,9-dimethyl-4-nitro-6-oxo-6,9-dihydro-3*H*-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**41**).

Table 1
In vitro antimycobacterial activity and cytotoxicity of compounds **13**, **15**, **20a,b,c**, **21a,c**, **23a**,**b,c**, **24a**,**c**, **29a**, **30a**, **31a, 32a**, **37** and **41**, against H37Rv.

Compound	MIC[a] (μM)	% Vero[b] cell viability at 100 μM
13	57.9	81.2 ± 4.4
15	>100	95.1 ± 3.6
20a	>100	91.2 ± 5.9
20b	27.8	89.5 ± 3.7
20c	>100	80.8 ± 4.9
21a	6.9	90.4 ± 2.1
21c	>100	85.7 ± 4.2
22a	>100	84.4 ± 4.3
22b	>100	79.3 ± 6.1
22c	>100	85.4 ± 5.3
23a	52.9	90.1 ± 5.9
23b	>100	94.2 ± 2.9
23c	>100	83.6 ± 5.0
24a	>100	90.2 ± 3.8
24c	>100	92.9 ± 4.8
29a	>100	92.0 ± 3.7
30a	6.6	94.3 ± 4.5
31a	>100	95.1 ± 2.8
32a	25.3	92.1 ± 6.0
37	50.3	87.7 ± 5.5
41	>100	88.8 ± 3.4
CPF[c]	1.5	95.2 ± 3.5

[a] Minimum inhibitory concentration.
[b] African kidney monkey cells (ATCC CCL-81).
[c] ciprofloxacin.

Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) used as internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; br s, broad singlet; dd, double doublet.

Mass spectra (MS) were performed on combined Liquid Chromatograph-Agilent 1100 series Mass Selective Detector (MSD). Analytical thin-layer chromatography (TLC) was performed on Merck silica gel F-254 plates. Pure compounds showed a single spot in TLC. For flash chromatography, Merck silica gel 60 was used with a particle size 0.040–0.063 mm (230–400 mesh ASTM). Elemental analysis were performed on a Perkin-Elmer 2400 instrument and the results were within ±0.4% of theoretical values.

5.1.1. Starting material and known intermediates

Polyvinylpyrrolidone with molecular weight 10 kDa (PVP10) was obtained from Sigma Aldrich (USA) and PVP K-30 (Kollidon®, molecular weight 40 kDa) was supplied by BASF (Germany). Hydroxypropyl methylcellulose K4M (HPMC) was obtained from Dow Chemicals Company (USA). Methyl-β-cyclodextrin (Cavasol® W7 M Pharma) was purchased from Wacker-Chemie GmbH (Germany). Eudragit® (L100–55) was generously donated by Degussa® (Germany). All other chemicals were of analytical grade.

Key intermediates *N*-(2-fluorophenyl)acetamide (**2**) and *N*-(2-fluoro-6-nitrophenyl)acetamide (**3a**) were prepared according to the procedures described in the literature [10]. 2-Fluoroaniline (**1**) was commercially available. Details of synthesis of each compound are following provided.

*5.1.2. 2-Fluoro-6-nitroaniline (**4**)*

3 g (0.0151 mol) of *N*-(2-fluoro-6-nitrophenyl)acetamide (**3a**) are dissolved in 30 ml of concentrated H_2SO_4 and brought to 50 °C for 2 h. After cooling, the solution is poured into an ice bath and an abundant precipitate is observed. After filtration, the acidic aqueus solution is extracted with diethyl ether. The pure compound is obtained through flash chromatography. Yield 74%. M.P. 47–48 °C. ^{1}H NMR (DMSO-d_6): δ 7.82 (d, 1H, J = 8.8 MHz, H-5), 7.42 (m, 1H, H-3), 7.25 (s, 2H, NH_2), 6.63 (m, 1H, H-4). GC/MS: 157 (M + H). Anal. Calcd for ($C_6H_5FN_2O_2$): C, 46.16; H, 3.23; F, 12.17; N, 17.94. Found: C, 46.18; H, 3.27; F, 12.15; N, 17.98.

*5.1.3. 3-Fluorobenzene-1,2-diamine (**5**)*

4.6 g (0.029 mol) of 2-fluoro-6-nitroaniline (**4**) are dissolved in 100 ml of ethanol and added with 0.46 g of 10% C/Pd. It is set to reduce by means of a hydrogenator; the theoretical amount of H_2 is rapidly absorbed at room temperature and at atmospheric pressure. By filtration the catalyst is removed, while the solvent is removed by evaporation: in this way a red oil is obtained which

Table 2
In silico predicted main pharmacokinetic parameters of compounds **13, 20b, 21a, 23a, 24a, 30a, 32a, and 37**. Data for ciprofloxacin are reported for comparison.

Cpd	MW[a]	HBA[b]	HBD[c]	logP[d]	logS[e]	PSA[f]	Rule of 5 violation	Drug likeness
Rule of 5	<500	≤10	≤5	≤5	≤5	–	≤1	
13	276.22	5	1	0.27	−3.33	71.64	0	0.31
20b	288.26	6	1	0.49	−2.65	78.82	0	0.29
21a	288.26	6	1	0.09	−2.81	79.28	0	−0.02
23a	302.29	6	1	0.56	−3.80	79.28	0	0.17
24a	274.23	6	2	0.00	−2.97	84.67	0	−0.36
30a	302.29	6	1	0.57	−3.18	78.85	0	0.30
32a	316.31	6	1	1.04	−4.18	78.86	0	0.23
37	318.35	6	1	1.02	−3.92	71.64	0	0.34
CPF[g]	331.35	4	2	1.29	−2.96	58.80	0	0.93

[a] Molecular weight.
[b] Number of hydrogen bond acceptors.
[c] Number of hydrogen bond donors.
[d] Logarithm of n-octanol/water partition coefficient.
[e] Logarithm of water solubility.
[f] Polar surface area.
[g] Ciprofloxacin.

Table 3
MIC values (μM) of **21a** against a set of Mtb strains obtained from clinical isolates. MDR = multi-drug resistant strains. MIC values of ciprofloxacin against resistant Mtb strains are also reported (in parenthesis) for comparison.

Mtb strain	SM[a]	INH[b]	RIF[c]	EMB[d]	CPF[e] (μM)	21a (μM)
H37Rv	S[f]	S	S	S	1.5	6.9
1358	S	R[g]	S	S	S	16
1670	S	S	S	S	R (16)	16
512 (MDR)	S	R	R	R	R (8)	8
7 (MDR)	R	R	R	R	S	4
231	S	S	S	S	S	4
1574	S	S	S	S	S	32
1777	S	S	S	S	S	8
147	S	S	S	S	S	8
1726	S	S	R	R	S	16
203	R	S	S	S	S	16
204	R	S	S	S	S	8
157	R	S	S	S	S	8
368	S	R	S	R	S	8
571 (MDR)	R	R	R	R	S	16
1120 (MDR)	R	R	R	R	S	4
1657	S	S	S	S	S	4
600	S	S	S	S	R (4)	16
1535	S	S	S	S	S	16
1567	S	S	S	S	S	32
952	S	S	R	S	S	32
1097	R	R	S	R	S	32

[a] Streptomycin.
[b] Isoniazid.
[c] Rifampicin.
[d] Ethambutol.
[e] Ciprofloxacin.
[f] Sensible.
[g] Resistant.

crystallizes in cold. Trough flash chromatography 3.2 g of clean diamine are collected as a dark violet oil. Yield 97%. Liquid oil. ^{1}H NMR (DMSO-d_6): δ 6.33 (m, 3H, H-4, H-5, H-6), 4.77 (s, 2H, NH_2), 4.33 (s, 2H, NH_2). GC/MS: 127 (M + H). Anal. Calcd for ($C_6H_7FN_2$): C, 57.13; H, 5.59; F, 15.06; N, 22.21. Found: C, 57.09; H, 5.61; F, 15.02; N, 22.24.

*5.1.4. 7-Fluoro-1H-benzo[d][1,2,3]triazole (**6**)*

3.66 g (0.029 mol) of 3-fluorobenzene-1,2-diamine (**5**) are dissolved in 200 ml of HCl 2 M and the solution is placed in an ice bath, bringing the temperature to 0 °C. A solution obtained by dissolving 4.08 g of $NaNO_2$ in 10 ml of H_2O is added drop by drop to over a period of ten minutes. After 12 h the formed precipitate is collected by filtration. The acidic solution was extracted with ethyl acetate,

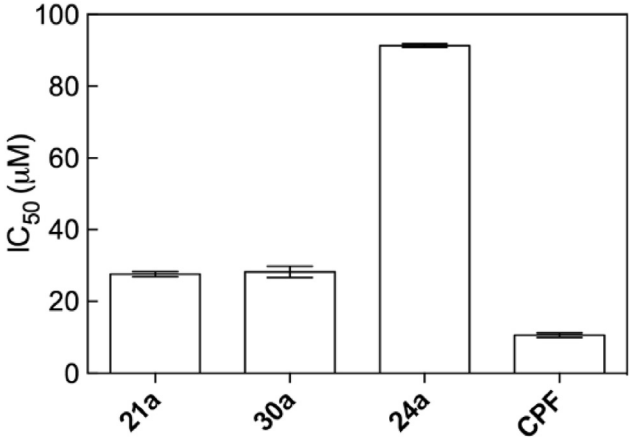


Fig. 3. IC_{50} values for Mtb DNA gyrase inhibition by compounds **21a**, **30a**, and **24a**. IC_{50} for ciprofloxacin (**CPF**) is also shown for comparison.

and the rough obtained compound is reunited to the precipitate. Trough flash chromatography we obtained the pure benzotriazole derivative as a beige solid compound. Yield 83%. M.p. 134–135 °C. ^{1}H NMR (DMSO-d_6): δ 7.68 (d, J = 8.6 Hz 1H, H-4)); 7.54 (m, 1H, H-5); 7.22 (m, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 168.50 (C), 144.79 (C), 142.26 (C), 125.80 (CH), 115.93 (CH), 108.70 (CH). GC/MS: 138. (M + H). Anal. Calcd for ($C_6H_4FN_3$): C, 52.56; H, 2.94; F, 13.86; N, 30.65. Found: C, 52.60; H, 2.99; F, 13.83; N, 30.60.

*5.1.5. General procedure for the preparation of fluoro-(1,2,3)-methyl-benzotriazoles (**7a-c**)*

1.5 g (0.0109 mol) of 7- fluoro-1*H*-benzo[*d*][1,2,3]triazole (**6**) are dissolved in 21.7 ml of NaOH 2 M (0.0434 mol). 1.61 ml (0.017 mol) 2.15 g of $(CH_3O)_2SO_2$ are added at room temperature. The solution is left to react for 18 h. An oil is formed which separates from the alkaline solution. The solution is extracted as it was first with ether, then with diethyl acetate, thus obtaining an oil (1.4 g) which contains the 3 isomers. Isomers were separated and purified by flash chromatography.

*5.1.5.1. 4-Fluoro-1-methyl-1H-benzo[d][1,2,3]triazole (**7a**).* Yield 38%. M.P. 79–80 °C. ^{1}H NMR: (DMSO) δ: 8.53 (d, J = 8.4 Hz, H-4); 7.68 (m, 1H, H-5); 7.22 (m, 1H, H-6); 4.33 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): 153.35 (C-3a), 150.82 (C-F), 135.02 (C-7a), 128.08 (CH),

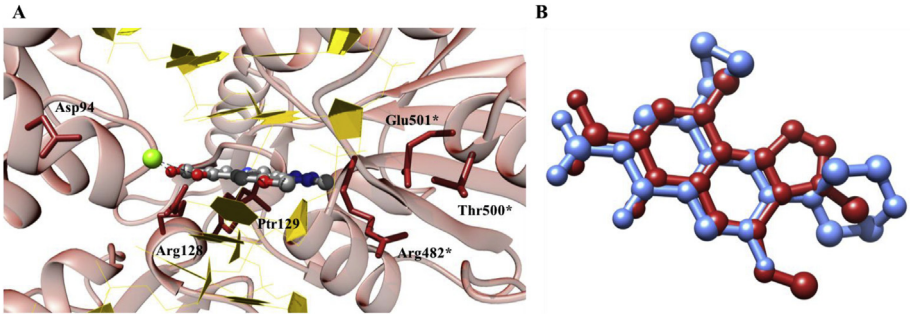


Fig. 4. (**A**) Putative binding mode of compound **21a** onto the Mtb DNA gyrase. The compound is shown as colored balls-and-sticks (gray, C; blue, N; red, O). The protein is represented as transparent firebrick ribbons. The protein residues mainly involved in **21a** binding are evidenced and labeled (see also main text for more details). Gyrase residues belonging to the subunit B (GyrB) are marked with an asterisk. Magnesium ion is depicted as a chartreuse sphere while the adjacent DNA bases are represented in gold. Coordination bonds are depicted as broken black lines. Hydrogen atoms, water molecules, ions and counterions are omitted for clarity. (**B**) Comparison of the docked conformations of compounds **21a** (firebrick) and **CPF** (corn-flower blue) in the Mtb DNA gyrase binding site.

108.21 (CH), 107.16 (CH), 34.35 (CH_3). GC/MS: 152 (M + H). Anal. Calcd for ($C_7H_6FN_3$): C, 55.63; H, 4.00; F, 12.57; N, 27.80. Found: C, 55.58; H, 4.03; F, 12.53; N, 27.87.

*5.1.5.2. 4-Fluoro-2-methyl-2H-benzo[d][1,2,3]triazole (**7b**).* Yield 25%. Liquid oil. ^{1}H NMR (DMSO-d_6), δ: 7.65 (d, J = 8.4 Hz 1H, H-7); 7.33 (m, 1H, H-6) 7.13 (m, 1H, H-5); 4.51 (s, 3H, N-CH_3). ^{13}C NMR (DMSO-d_6): 154.78 (C); 146.51 (C); 134.52 (C); 126.31 (CH); 113.87 (CH); 109.39 (CH); 43.27 (CH_3). GC/MS:152 (M + H). Anal. Calcd for ($C_7H_6FN_3$): C, 55.63; H, 4.00; F, 12.57; N, 27.80. Found: C, 55.59; H, 4.06; F, 12.61; N, 27.75.

*5.1.5.3. 7-Fluoro-1-methyl-1H-benzo[d][1,2,3]triazole (**7c**).* Yield 33%. M.P. 65–66 °C. ^{1}H -NMR: ($CDCl_3$) δ 7.81 (d, J = 8.4 Hz, 1H, H-4); 7.27 (m, 1H, H-5); 7.12 (t, J = 9.2 Hz, 1H, H-6); 4.45 (s, 3H, N-CH_3). ^{13}C NMR ($CDCl_3$): 150.29 (C-F); 149.12 (C-3a), 145.32 (C-7a); 124.23 (CH); 115.70 (CH); 111.59 (CH); 36.27 (CH_3). GC/MS: 152 (M + H). Anal. Calcd for ($C_7H_6FN_3$): C, 55.63; H, 4.00; F, 12.57; N, 27.80. Found: C, 55.65; H, 4.01; F, 12.53; N, 27.82.

*5.1.6. Synthesis of fluorine-methyl-triazolo derivatives (**8a-15**)*

*5.1.6.1. 7-Fluoro-1-methyl-4-nitro-1H-benzo[d][1,2,3]triazole (**8a**).* 4.2 g (0.0277 mol) 7-fluoro-1-methyl-1*H*-benzotriazole (**7c**) are dissolved in 54.6 ml of H_2SO_4 conc. To the acidic solution a mixture consisting of 10.3 g of KNO_3 in 54.6 ml of H_2SO_4 conc. is added drop by drop at room temperature. The temperature is then brought to 50 °C and left under stirring. After an hour we let the solution return to room temperature and then poured into an ice bath. A light yellow precipitate is formed and subsequently purified by chromatography. Yield 65%. M.P. 115–116 °C. ^{1}H NMR: ($CDCl_3$) δ: 8.31 (dd, 1H, H-5); 7.26 (t, J = 9.2 Hz, 1H, H-6); 4.56 (s, 3H, N-CH_3). ^{13}C NMR: ($CDCl_3$) δ: 160.22 (C), 153.87 (C), 133.21 (C), 129.75 (C), 126.39 (CH), 107.86 (CH), 41.01 (CH_3), GC/MS: 197 (M + H). Anal. Calcd for ($C_7H_5FN_4O_2$): C, 42.87; H, 2.57; F, 9.69; N, 28.57. Found: C, 42.83; H, 2.61; F, 9.72; N, 28.55.

*5.1.6.2. 4-Fluoro-2-methyl-7-nitro-2H-benzo[d][1,2,3]triazole (**8b**).* 2.06 g (0.0136 mol) of 4-fluoro-2-methyl-2*H*-benzo[*d*][1,2,3]triazole (**7b**) are dissolved in 5 ml of glacial CH_3COOH and brought to reflux. To the acidic solution were added 1.85 ml (0.053 mol) 2.50 g of fuming HNO_3 drop by drop. The solution was left stirring for 24 h. Finally, the solution is cooled with an ice bath: a solid mass is formed. 2.15 g of a pale yellow solid is filtered and subsequently purified by chromatography. Yield: 51%. M.P. 52–53 °C. ^{1}H NMR ($CDCl_3$), δ: 8.53 (dd, 1H, H-6); 7.52 (t, J = 9.2 Hz, 1H, H-5); 4.66 (s, 3H, N-CH_3). ^{13}C NMR: ($CDCl_3$) δ: 159.41 (C), 154.00 (C), 140.10 (C), 134.32 (C), 126.48 (CH), 109.20 (CH), 44.38 (CH_3). GC/MS: 197 (M + H). Anal. Calcd for ($C_7H_5FN_4O_2$): C, 42.87; H, 2.57; F, 9.69; N, 28.57. Found: C, 42.91; H, 2.55; F, 9.71; N, 28.53.

*5.1.6.3. 7-Fluoro-1-methyl-4-nitro-1H-benzo[d][1,2,3]triazole (**8c**).* 2.00 g (0.013 mol) of 4-fluoro-1-methyl-1*H*-benzo[*d*][1,2,3]triazole (**7c**) are dissolved in 26 ml of H_2SO_4 conc. To the acidic solution a mixture consisting of 4.92 g of KNO_3 in 26 ml of H_2SO_4 conc. is added drop by drop at room temperature. The temperature is then brought to 50 °C and left under stirring. After an hour we let the solution return to room temperature and then poured into an ice bath. A light yellow precipitate is formed and subsequently purified by chromatography. Yield 53%. M.P. 83–84 °C. ^{1}H NMR (DMSO-d_6), δ: 8.42 (dd, 1H, H-6); 7.43 (t, J = 9.2 Hz, 1H, H-5); 4.83 (s, 3H, N-CH_3). ^{13}C NMR: ($CDCl_3$) δ: 159.98 (C), 154.58 (C), 131.09 (C), 129.05 (C), 127.53 (CH), 108.48 (CH), 40.09 (CH_3). GC/MS: 197 (M + H). Anal. Calcd for ($C_7H_5FN_4O_2$): C, 42.87; H, 2.57; F, 9.69; N, 28.57. Found: C, 42.90; H, 2.55; F, 9.71; N, 28.54.

*5.1.6.4. 7-Fluoro-1-methyl-1H-benzo[d][1,2,3]triazol-4-amine (**9**).* 1.71 g (8.72×10^{-3} mol) of 7-fluoro-1-methyl-4-nitro-1*H*-benzo[*d*][1,2,3]triazole (**8**) are dissolved in 105 ml of 37% HCl. Then 19.5 g (8.38×10-2 mol, ≈ 10 equivalents) of $SnCl_2$ H_2O are added. The reaction temperature is then brought to reflux and from the moment the solution becomes clear, one hour is expected. After solution returns to room temperature, it is neutralized with 50% NaOH. The solution obtained now contains abundant hydroxide which is filtered. The aqueous solution, neutralized and violet, are further basified until yellowish coloration. The mothers are extracted with ethyl ether, and the solid is also thoroughly washed with the same solvent. By evaporation of the organic phase we get pure amine (**9**). Yield 70%. M.P. 118–119 °C. ^{1}H NMR: ($CDCl_3$, δ ppm): 7.265 (s, 2H, NH_2), 6.922 (dd, 1H, J = 10.6, J = 10.4, H-5), 6.325 (dd, 1H, J = 3.2, J = 8.4, H-6), 4.397 (s, 3H, CH_3).). ^{13}C NMR: ($CDCl_3$) δ: 155.68 (C), 150.18 (C), 133.79 (C),. 130.07 (C), 117.38 (CH), 115.88 (CH), 35.12 (CH_3). GC/MS: 167 (M + H). Anal. Calcd for ($C_7H_7FN_4$): C, 50.60; H, 4.25; F, 11.43; N, 33.72. Found: C, 50.63; H, 4.21; F, 11.47; N, 33.76.

*5.1.6.5. Diethyl 2-(((7-fluoro-1-methyl-1H-benzo[d][1,2,3]triazol-4-yl)amino)methylene)malonate (**10**).* 1.63 g (9.81×10^{-3} mol) of 7-fluoro-1-methyl-1*H*-benzo[*d*][1,2,3]triazol-4-amine (**9**) are suspended in 35 ml of Dowtherm. 2.33 g (1.08×10^{-2} mol) 2.16 ml of **d**iethyl (ethoxymethylene)malonate are then added. The reaction temperature is then brought up to 150 °C and allowed to react for 17 h. After returning the solution to room temperature, it is diluted

with hexane, obtaining copious precipitation. The product is then filtered to obtain a gray solid. The solid is abundantly washed with ethyl ether, obtaining the pure product. Yield 90%. M.P. 76–77 °C. ^{1}H NMR: ($CDCl_3$) δ: 11.623 (d, J = 13, 1H, NH), 9.322 (d, 1H, J = 13, CH), 6.922 (m, 2H, H-6, H-5), 4.307 (m, 4H, 2 CH_2), 1.404 (m, 6H, 2 CH_3). ^{13}C NMR: ($CDCl_3$) δ: 171.22 (2 C), 155.12 (C), 151.23 (CH), 149.73 (C), 134.34 (C),. 130.31 (C), 117.09 (CH), 115.74 (CH), 97.36 (C), 59.82 (2 CH_2), 35.36 (CH_3), 14.25 (2 CH_3).GC/MS: 337 (M + H). Anal. Calcd for ($C_{15}H_{17}FN_4O_4$): C, 53.57; H, 5.10; F, 5.65; N, 16.66. Found: C, 53.60; H, 5.07; F, 5.68; N, 16.70.

*5.1.6.6. Ethyl 4-fluoro-3-methyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**11**).* 1.70 g (5.05×10^{-3} mol) of 2-(((7-fluoro-1-methyl-1*H*-benzo[*d*][1,2,3]triazol-4-yl)amino) methylene)malonate (**10**) are poured into 40 ml of boiling Dowtherm. It is left to react for 15 min, after which the solution is returned to rt. It is diluted with 40 ml of hexane, obtaining copious precipitation. The obtained gelatinous product is then shelled with abundant ethyl ether, obtaining pure compound as a dusty brown solid. Yield 84%. M.P. 254–256 °C. ^{1}H NMR: ($CDCl_3$) δ: 8.48 (s, 2H, NH_2), 8.21 (s, 1H, H-9), 7.87 (d, 1H, J = 11.4, H-6), 4.50 (s, 3H, CH_3), 4.28 (q, 2H, CH_2-CH_3), 1.33 (t, 3H, CH_2-CH_3). ^{13}C NMR: ($CDCl_3$) δ: 172.17 (C), 165.31 (C), 153.89 (C), 146.39 (CH), 143.11 (C), 134.36 (C), 130.49 (C), 129.22 (C), 112.07 (C), 102.46 (CH), 61.21 (CH_2), 35.60 (CH_3), 14.12 (CH_3). GC/MS: 291 (M + H). Anal. Calcd for ($C_{13}H_{11}FN_4O_3$): C, 53.80; H, 3.82; F, 6.55; N, 19.30. Found: C, 53.77; H, 3.85; F, 6.36; N, 19.27.

*5.1.6.7. Ethyl 4-fluoro-3,9-dimethyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**12**).* 0.83 g (2.8×10^{-3} mol) of ethyl 4-fluoro-3-methyl-6-oxo-6,9-dihydro-3*H*-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**11**) are added to a suspension of 0.63 g (1.12×10^{-2} mol) of KOH in 20 ml of DMSO at room temperature. After 30 min, 1.58 g (1.12×10^{-2} mol) 0.7 ml of CH_3I are added and the reaction is stopped for 1 h. The solution is then diluted with 3 ml of water, obtaining complete precipitation. The solid is filtered, washed with water and dried, thus obtaining 0.65 g of a pure compound. Yield: 75%. M.P.: 217–219 °C. ^{1}H NMR: ($CDCl_3$) δ: 8.50 (s, 1H, H-8), 8.27 (d, 1H, J = 11.4, H-5), 4.59 (s, 3H, N3-CH_3), 4.54 (s, 3H, N9-CH_3), 4.42 (q, 2H, CH_2-CH_3), 1.43 (t, 3H, CH_3-CH_2). ^{13}C NMR: ($CDCl_3$) δ: 176.07 (C), 164.88 (C), 153.17 (C), 147.03 (CH), 143.86 (C), 134.36 (C), 131.02 (C), 129.62 (C), 112.41 (C), 103.21 (CH), 61.34 (CH_2), 45.60 (CH_3), 35.88 (CH_3), 14.36 (CH_3). GC/MS: 305 (M + H). Anal. Calcd for ($C_{14}H_{13}FN_4O_3$): C, 55.26; H, 4.31; F, 6.24; N, 18.41. Found: C, 55.22; H, 4.37; F, 6.19; N, 18.46.

*5.1.6.8. 4-Fluoro-3,9-dimethyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**13**).* 0.30 g (9.86×10^{-4} mol) of Ethyl 4-fluoro-3,9-dimethyl-6-oxo-6,9-dihydro-3*H*-[1,2,3]triazolo [4,5-h]quinoline-7-carboxylate (**12**) are dissolved in 20 ml of NaOH 2 M. The reaction temperature is brought to 100 °C and allowed to react for one hour. Returned to room temperature, the solution is acidified with HCl 6 M, obtaining complete precipitation. The gelatinous solid is filtered, washed with plenty of water, and dried, obtaining the pure product. Yield: 82%. M.P. > 300 °C. ^{1}H NMR: (Acetone) δ: 8.95 (s, 1H, H-8), 8.13 (d, 1H, H-5), 4.83 (s, 3H, CH_3), 4.64 (s, 3H, CH_3). ^{13}C NMR: ($CDCl_3$) δ: 175.44 (C), 166.27 (C), 154.01 (C), 151.83 (CH), 144.51 (C), 134.92 (C), 131.42 (C), 129.88 (C), 112.90 (C), 102.89 (CH), 45.38 (CH_3), 35.95 (CH_3). GC/MS: 277 (M + H). Anal. Calcd for ($C_{12}H_9FN_4O_3$): C, 52.18; H, 3.28; F, 6.88; N, 20.28. Found: C, 52.15; H, 3.33; F, 6.83; N, 20.31.

*5.1.6.9. Ethyl 9-ethyl-4-fluoro-3-methyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**14**).* 0.82 g (2.8×10^{-3} mol) of Ethyl 4-fluoro-3-methyl-6-oxo-6,9-dihydro-3*H*-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**11**) are dissolved in 10 ml of DMFa and brought to 0 °C. 0.135 g (5.63×10^{-3} mol) of NaH are added and allowed to react for 10 min 0.5 ml (6.25×10^{-3} mol) 0.975 g of CH_3CH_2I are then dripped. It is left to react for 3 h, after which the solution is poured into ice. The aqueous solution is extracted with ethyl acetate. The solid product obtained is purified by flash chromatography with $CHCl_3$ to eliminate traces of the starting product. M.P.: 196–197 °C. Yield: 51%. ^{1}H NMR: ($CDCl_3$) δ: 8.53 (s, 1H, H-8), 8.28 (d, 1H, J = 11, H-5), 5.11 (q, 2H, N9-CH_2-CH_3), 4.54 (s, 3H, N3-CH_3), 4.43 (q, 2H, O-CH_2-CH_3), 1.61 (t, 3H, N9-CH_2-CH_3), 1.44 (t, 3H, O-CH_2-CH_3). ^{13}C NMR: ($CDCl_3$) δ: 175.87 (C), 164.59 (C), 152.84 (C), 147.36 (CH), 144.09 (C), 134.83 (C), 131.27 (C), 129.47 (C), 111.32 (C), 105.19 (CH), 61.47 (CH_2), 52.06 (CH_2), 35.88 (CH_3), 14.41 (CH_3), 14,21 (CH_3).GC/MS: 318 (M + H). Anal. Calcd for ($C_{15}H_{15}FN_4O_3$) C, 56.60; H, 4.75; F, 5.97; N, 17.60. Found: C, 56.57; H, 4.78; F, 6.01; N, 17.3.

*5.1.6.10. 9-Ethyl-4-fluoro-3-methyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**15**).* 0.09 g (2.83 10^{-4} mol) of. Ethyl 9-ethyl-4-fluoro-3-methyl-6-oxo-6,9-dihydro-3*H*-[1,2,3] triazolo[4,5-h]quinoline-7-carboxylate (**14**) are solubilized in 5 ml of DMSO and then 0.048 g (4.24 10^{-4} mol) of Potassium *tert*-butoxide are added. It is refluxed and allowed to react for 3 h. After one hour the reaction was switched off and let to return at room temperature. 5 ml of water are added and the solution is acidified with HCl 2 M, obtaining complete precipitation. The solid is filtered, abundantly washed with water, washed with ethyl ether and finally dried to obtain a pure gray compound. M.P.: 266–267 °C. Yield: 73%. ^{1}H NMR: ($CDCl_3$) δ: 9.12 (s, 1H, H8), 8.03 (s, 1H, H5), 5.22 (q, 2H, CH_2), 4.52 (s, 3H, CH_3), 1.49 (t, 3H, CH_3). ^{13}C NMR: ($CDCl_3$) δ: 176.27 (C), 167.19 (C), 155.12 (C), 150.39 (CH), 144.16 (C), 135.31 (C), 132.42 (C), 129.67 (C), 113.22 (C), 103.80 (CH), 51.89 (CH_2), 35.68 (CH_3), 14.80 (CH_3). GC/MS: 291 (M + H). Anal. Calcd for ($C_{13}H_{11}FN_4O_3$): C, 53.80; H, 3.82; F, 6.55; N, 19.30. Found: C, 53.83; H, 3.79; F, 6.51; N, 19.36.

*5.1.7. General procedure for the preparation of the intermediate methoxy-methyl-nitro-benzotriazoles (**16a-c**) and ethoxy-methyl-nitro-benzotriazole (**25a**)*

0.5 g of the adequate fluoro-methyl-nitro-benzotriazole (**8a-c**) are suspended in 20 ml of methanol or ethanol with 1 ml of Et_3N. The solution is refluxed for from 4 to 20 h. A solid precipitate from the solution. After cooling down the solution, the precipitate is filtered and then abundantly washed with ethyl ether, obtaining the pure product.

*5.1.7.1. 7-Methoxy-1-methyl-4-nitro-1H-benzo[d][1,2,3]triazole (**16a**).* Solvent: methanol. Time reaction: 4 h. M.P.:116–117 °C. Yield: 53%. ^{1}H NMR: ($CDCl_3$) δ: 8.28 (d, 1H, H-6); 6.82 (d, 1H, H-5); 4.54 (s, 3H, NCH_3); 4.14 (s, 3H, OCH_3). GC/MS: 209 (M + H). Anal. Calcd for ($C_8H_8N_4O_3$) C, 46.16; H, 3.87; N, 26.91. Found: C, 46.20; H, 3.85; N, 26.88.

*5.1.7.2. 4-Methoxy-2-methyl-7-nitro-2H-benzo[d][1,2,3]triazole (**16b**).* Solvent: methanol. Time reaction: 20 h. M.P.:215–216 °C. Yield: 75%. ^{1}H NMR: ($CDCl_3$) δ: 8.45 (d, J = 8, 1H, H-6); 6.75 (d, J = 8.4, 1H, H-5); 4.64 (s, 3H, NCH_3); 4.19 (s, 3H, OCH_3). GC/MS: 209 (M + H). Anal. Calcd for ($C_8H_8N_4O_3$) C, 46.16; H, 3.87; N, 26.91. Found: C, 46.18; H, 3.90; N, 26.93.

*5.1.7.3. 4-Methoxy-1-methyl-7-nitro-1H-benzo[d][1,2,3]triazole (**16c**).* Solvent: methanol. Time reaction: 10 h. M.P.:72–73 °C. Yield: 81%. ^{1}H NMR: ($CDCl_3$) δ: 8.40 (d, J = 9.2 Hz, 1H, H6)), 6.77 (d, J = 8.6 Hz, 1H, H5); 4.62 (s, 3H, NCH_3); 4.24 (s, 3H, OCH_3). GC/MS: 209 (M + H). Anal. Calcd for ($C_8H_8N_4O_3$) C, 46.16; H, 3.87; N, 26.91.

Found: C, 46.17; H, 3.92; N, 26.87.

*5.1.7.4. 4-Ethoxy-1-methyl-7-nitro-1H-benzo[d][1,2,3]triazole (**25a**).* Solvent: ethanol. Time reaction: 18 h. M.P.:78–79 °C. Yield: 56%. ^{1}H NMR: ($CDCl_3$) δ: 8.27 (d, 1H, H5); 6.78 (d, 1H, H6); 4.55 (s, 3H, N-CH_3); 4.36 (q, 2H, CH_2CH_3); 1.61 (t, 3H, CH_3CH_2).). GC/MS: 223 (M + H). Anal. Calcd for ($C_9H_{10}N_4O_3$) C, 48.65; H, 4.54; N, 25.21. Found: C, 48.67; H, 4.56; N, 25.19.

*5.1.8. General procedure for the preparation of the intermediate methoxy-methyl-benzotriazol-amines (**17a-c**) and ethoxy-methyl-nitro-benzotriazole (**26a**)*

1 g of the adequate methoxy-methyl-nitro-benzotriazoles (**16a-c**) or ethoxy-methyl-nitro-benzotriazole (**25a**) is dissolved in 100 ml of THF or $CHCl_3$ and added with 0.1 g of 10% C/Pd. It is set to reduce by means of a hydrogenator; the theoretical amount of H_2 is rapidly absorbed at room temperature and at atmospheric pressure. By filtration the catalyst is removed, while the solvent is removed by evaporation: in this way a crystal solid is obtained, which is crystalized in ethanol.

*5.1.8.1. 7-Methoxy-1-methyl-1H-benzo[d][1,2,3]triazol-4-amine (**17a**).* Solvent: $CHCl_3$. Time reaction: 3 h. M.P.:119–120 °C. Yield: 77%. ^{1}H NMR: ($CDCl_3$) δ: 6.57 (d, J = 8, 1H, H-6); 6.37 (d, J = 8.2, 1H, H-5); 4.43 (s, 3H, NCH_3); 3.89 (s, 3H, OCH_3). GC/MS: 179 (M + H). Anal. Calcd for ($C_8H_{10}N_4O$) C, 53.92; H, 5.66; N, 31.44. Found C, 53.94; H, 5.68; N, 31.40.

*5.1.8.2. 7-Methoxy-2-methyl-2H-benzo[d][1,2,3]triazol-4-amine (**17b**).* Solvent: THF. Time reaction: 4 h. M.P.:217–218 °C. Yield: 75%. ^{1}H NMR: ($CDCl_3$) δ: 8.45 (d, J = 8, 1H, H-6); 6.75 (d, J = 8.4, 1H, H-5); 4.64 (s, 3H, NCH_3); 4.19 (s, 3H, OCH_3). GC/MS: 179 (M + H). Anal. Calcd for ($C_8H_{10}N_4O$) C, 53.92; H, 5.66; N, 31.44. Found C, 53.90; H, 5.67; N, 31.39.

*5.1.8.3. 4-Methoxy-1-methyl-1H-benzo[d][1,2,3]triazol-7-amine (**17c**).* Solvent: THF. Time reaction: 4 h. M.P.: 56–57 °C. Yield: 88%. ^{1}H NMR: ($CDCl_3$) δ: 6.75 (s, 2H, NH_2); 6.61 (d, 1H, H6); 6.43 (d, 1H, H5); 4.51 (s, 3H, NCH_3); 3.98 (s, 3H, OCH_3). GC/MS: 179 (M + H). Anal. Calcd for ($C_8H_{10}N_4O$) C, 53.92; H, 5.66; N, 31.44. Found C, 53.89; H, 5.65; N, 31.45.

*5.1.8.4. 7-Ethoxy-1-methyl-1H-benzo[d][1,2,3]triazol-4-amine (**26a**).* Solvent: $CHCl_3$. Time reaction: 2 h. M.P.: 132–133 °C. Yield: 69%. ^{1}H NMR: ($CDCl_3$) δ: 6.57 (d, 1H, H5); 6.35 (d, 1H, H6); 4.44 (s, 3H, N-CH_3), 4.27 (s, 2H, NH_2); 4.09 (q, 2H, CH_2CH_3); 1.47 (t, 3H, CH_3CH_2). GC/MS: 193 (M + H). Anal. Calcd for ($C_9H_{12}N_4O$) C, 56.24; H, 6.29; N, 29.15. Found C, 56.20; H, 6.32; N, 29.14.

*5.1.9. General procedure for the preparation of the intermediate diethyl 2-[(methoxy-methyl-benzotriazol-yl)amino)methylene] malonate derivatives (**18a-c**) and diethyl 2-(((7-ethoxy-1-methyl-1H-benzo[d][1,2,3]triazol-4-yl)amino)methylene)malonate (**27a**)*

1 g of methoxy-methyl-benzotriazol-amines (**17a-c**) or ethoxy-methyl-nitro-benzotriazole (**26a**). are suspended in 35 ml of Dowtherm. Diethyl (ethoxymethylene)malonate is added to the solution in a molar ratio (1:1,1). The reaction temperature is then brought up to 150 °C and allowed to react for 17 h. After returning the solution to room temperature, it is diluted with hexane, obtaining copious precipitation. The product is then filtered to obtain a solid. The solid is abundantly washed with ethyl ether, obtaining the pure product.

*5.1.9.1. Diethyl 2-(((7-methoxy-1-methyl-1H-benzo[d][1,2,3]triazol-4-yl)amino)methylene)malonate (**18a**).* M.P.: 130–131 °C. Yield: 63%. ^{1}H NMR: ($CDCl_3$) δ: 11.55 (d, J = 14, 1H, NH), 9.72 (d, J = 14, 1H, CH), 6.90 (d, J = 8, 1H, H6), 6.70 (d, J = 9, 1 H, H6), 4.48 (s, 1H, NCH_3), 4.32 (q, 4H, 2 CH_2-CH_3), 3.98 (s, 3H, OCH_3), 1.39 (q, 6H, 2 CH_3-CH_2). GC/MS: 349 (M + H). Anal. Calcd for ($C_{16}H_{20}N_4O_5$): C, 55.17; H, 5.79; N, 16.08. Found: C, 55.19; H, 5.82; N, 16.10.

*5.1.9.2. Diethyl 2-(((7-methoxy-2-methyl-2H-benzo[d][1,2,3]triazol-4-yl)amino)methylene)malonate (**18b**).* M.P.: 118–119 °C. Yield: 66%. ^{1}H NMR: ($CDCl_3$) δ: 11.44 (d, 1H, NH), 9.24 (d, 1H, CH), 6.95 (d, 1H, H5), 6.59 (d, 1H, H6), 4.53 (s, 3H, OCH_3), 4.29 (m, 4H, 2 CH_2CH_3), 4.03 (s, 3H, CH_3), 1.41 (q, 6H, 2 CH_3CH_2). GC/MS: 349 (M + H). Anal. Calcd for ($C_{16}H_{20}N_4O_5$): C, 55.17; H, 5.79; N, 16.08. Found: C, 55.15; H, 5.74; N, 16.11.

*5.1.9.3. Diethyl 2-(((4-methoxy-1-methyl-1H-benzo[d][1,2,3]triazol-7-yl)amino)methylene)malonate (**18c**).* M.P.: 115–116 °C. Yield: 70%. ^{1}H NMR: ($CDCl_3$) δ: 11.42 (d, 1H, J = 12.8 Hz, NH), 8.35 (d, 1H, J = 12.6 Hz, CH =), 7.17 (d, 1H, J = 8 Hz, H5), 6.46 (d, 1H, J = 8 Hz, H6), 4.51 (s, 3H, OCH_3), 4.38–4.17 (m, 4H, 2 CH_2CH_3), 4.09 (s, 3H, CH_3), 1.43–1.28 (m, 6H, 2 CH_3CH_2). GC/MS: 349 (M + H). Anal. Calcd for ($C_{16}H_{20}N_4O_5$): C, 55.17; H, 5.79; N, 16.08. Found: C, 55.14; H, 5.77; N, 16.09.

*5.1.9.4. Diethyl 2-(((7-ethoxy-1-methyl-1H-benzo[d][1,2,3]triazol-4-yl)amino)methylene)malonate (**27a**).* M.P.: 141–142 °C. Yield: 57%. ^{1}H NMR: ($CDCl_3$) δ: 11.54 (d, 1H, NH), 9.35 (d, 1H, CH), 6.88 (d, 1H, H5), 6.68 (d, 1H, H6), 4.49 (s, 3H, N-CH_3), 4.14–4.38 (m, 6H, CH_2CH_3), 1.33–1.58 (m, 9H, CH_3CH_2). GC/MS: 363 (M + H). Anal. Calcd for ($C_{17}H_{22}N_4O_5$): C, 56.35; H, 6.12; N, 15.46. Found: C, 56.38; H, 6.09; N, 15.51.

*5.1.10. General procedure for the preparation of the intermediate ethyl-methoxy-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylate derivatives (**19a-c**) and ethyl 4-ethoxy-3-methyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**28a**)*

1 g of the appropriate diethyl 2-[(methoxy-methyl-benzotriazol-yl)amino)methylene]malonate derivatives (**18a-c**) or diethyl 2-(((7-ethoxy-1-methyl-1H-benzo[d][1,2,3]triazol-4-yl) amino)methylene)malonate (**27a**) are poured into 40 ml of boiling Dowtherm. It is left to react for 15 min, after which the solution is returned to rt. The solution is diluted with 40 ml of hexane, obtaining copious precipitation. The obtained product is then shelled with abundant ethyl ether. Finally, the obtained compound is crystallized from ethanol.

*5.1.10.1. Ethyl 4-methoxy-3-methyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**19a**).* M.P.: 263–264 °C. Yield: 88%. ^{1}H NMR: ($CDCl_3$) δ: 4 12.32 (s, 1H, NH), 9.19 (s, 1H, $H_{quinolone}$), 7.38 (s, 1H, H6), 4.56 (m, 5H, NCH_3 + CH_2-CH_3), 0.123 (s, 3H, OCH_3), 1.49 (q, 3H, CH_3-CH_2). GC/MS: 303 (M + H). Anal. Calcd for ($C_{14}H_{14}N_4O_4$): C, 55.63; H, 4.67; N, 18.53. Found: C, 55.66; H, 4.70; N, 18.51.

*5.1.10.2. Ethyl 4-methoxy-2-methyl-6-oxo-6,9-dihydro-2H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**19b**).* M.P.: 228–229 °C. Yield: 83%. ^{1}H NMR: ($CDCl_3$) δ: 9.12 (s, 1H, H8), 7.29 (s, 1H, H5), 4.59 (s, 3H, OCH_3), 4.51 (m, 2H, CH_2CH_3), 4.16 (s, 3H, CH_3), 1.50 (t, 3H, CH_3CH_2). GC/MS: 303 (M + H). Anal. Calcd for ($C_{14}H_{14}N_4O_4$): C, 55.63; H, 4.67; N, 18.53. Found: C, 55.63; H, 4.65; N, 18.55.

*5.1.10.3. Ethyl 4-methoxy-1-methyl-6-oxo-6,9-dihydro-1H-[1,2,3]triazolo [4,5-h]quinoline-7-carboxylate (**19c**).* M.P.: 204–205 °C. Yield: 73%. ^{1}H NMR: ($CDCl_3$) δ: 12.25 (s, 1H, NH), 9.07 (s, 1H, H8), 7,26 (s, 1H, H5), 4.83 (s, 3H, OCH_3), 4.54 (m, 2H, CH_2CH_3), 4.18 (s, 3H, CH_3),

1.49 (t, 3H, CH_3CH_2). Anal. Calcd for ($C_{14}H_{14}N_4O_4$): C, 55.63; H, 4.67; N, 18.53. Found: C, 55.60; H, 4.71; N, 18.50.

*5.1.10.4. Ethyl 4-ethoxy-3-methyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**28a**).* M.P.: 280–281 °C. Yield: 97%. ^{1}H NMR: ($CDCl_3$) δ: 12.28 (s, 1H, NH), 9.19 (s, 1H, H8), 7.38 (s, 1H, H5), 4.60 (s, 3H, N-CH_3), 4.30–4.55 (m, 4H, CH_2CH_3), 1.46–1.64 (m, 6H, CH_3CH_2). GC/MS: 317 (M + H). Anal. Calcd for ($C_{15}H_{16}N_4O_4$): 56.96; H, 5.10; N, 17.71. Found: 56.99; H, 5.08; N, 17.73.

*5.1.11. General procedure for the preparation of methoxy-dimethyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**20a**), 4-methoxy-2,9-dimethyl-6-oxo-6,9-dihydro-2H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**20b**), ethyl 4,6-dimethoxy-1-methyl-1H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**20c**) and ethyl 4-methoxy-3,9-dimethyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**29a**)*

1.00 g of the appropriate ethyl-methoxy-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylate derivatives (**19a-c**) or ethyl 4-ethoxy-3-methyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo [4,5-h]quinoline-7-carboxylate (**28a**). are added to a suspension of 0.76 g of KOH in 20 ml of DMSO at room temperature. After 30 min, 1.90 g (1.33×10^{-2} mol) 0.83 ml of CH_3I are added and the reaction is stopped for 1 h. The solution is then diluted with 3 ml of water, obtaining complete precipitation. The solid is filtered, washed with water and dried. Pure compounds were crystalized from ethanol.

*5.1.11.1. Ethyl 4-methoxy-3,9-dimethyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**20a**).* M.P.: 256–257 °C. Yield: 59%. ^{1}H NMR: (DMSO-d_6) δ: 8.56 (s, 1H, H9), 7.53 (s, 1H, H6), 4.47 (s, 3H, N3-CH_3), 4.30 (s, 3H, OCH_3), 4.25 (q, 2H, CH_2), 4.03 (s, 3H, N9-CH_3), 1.31 (t, 3H, J = 7.4, CH_3-CH_2). ^{13}C NMR: (DMSO-d_6) δ: 171.10 (C), 164.55 (C), 148.40 (CH), 143.50 (C), 139.23 (C), 128.64 (C), 127.48 (C), 125.65 (C), 111.18 (C), 101.77 (CH), 59.82 (CH_2), 56.38 (CH_3), 51.32 (CH_3), 33.05 (CH_3), 14.25 (CH_3). GC/MS: 317 (M + H). Anal. Calcd for ($C_{15}H_{16}N_4O_4$): C, 56.96; H, 5.10; N, 17.71. Found: C, 57.00; H, 5.12; N, 17.69.

*5.1.11.2. 4-Methoxy-2,9-dimethyl-6-oxo-6,9-dihydro-2H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**20b**).* M.P.> 300 °C. Yield: 41%. ^{1}H NMR: ($CDCl_3$) δ: 7.91 (s, 1H, H8), 7.22 (s, 1H, H5), 4.64 (s, 3H, OCH_3), 4.59 (s, 3H, CH_3), 4.17 (s, 3H, CH_3). GC/MS: 289 (M + H). Anal. Calcd for ($C_{13}H_{12}N_4O_4$): C, 54.17; H, 4.20; N, 19.44. Found: C, 54.19; H, 4.18; N, 19.46.

*5.1.11.3. Ethyl 4,6-dimethoxy-1-methyl-1H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**20c**).* M.P.: 146–147 °C. Yield: 80%. ^{1}H NMR: (DMSO-d_6) δ: 8.98 (s, 1H, H8), 7.23 (s, 1H, H5), 4.72 (s, 3H, CH_3), 4.33 (q, 2H, $J = 6.8$ Hz, CH_2), 4.12 (s, 3H, CH_3), 4.10 (s, 3H, CH_3), 1.39 (t, 3H, J = 7.4, CH_3). ^{13}C NMR: (DMSO-d_6) δ: 164.68 (C), 162.13 (C), 149.77 (C), 148.33 (CH), 139.99 (C), 136.25 (C), 130.45 (C), 123.15 (C), 115.77 (C), 94.51 (CH), 62.76 (CH_3), 61.59 (CH_2), 57.46 (CH_3), 37.58 (CH_3), 13.95 (CH_3). GC/MS: 317 (M + H). Anal. Calcd for ($C_{15}H_{16}N_4O_4$): C, 56.96; H, 5.10; N, 17.71. Found: C, 56.98; H, 5.09; N, 17.74.

*5.1.11.4. Ethyl 4-ethoxy-3,9-dimethyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**29a**).* M.P.:228–229 °C. Yield: 94%. ^{1}H NMR: ($CDCl_3$) δ: 8.40 (s, 1H, H8), 7.45 (s, 1H, H5), 4.65 (s, 3H, CH_3), 4.57 (s, 3H, CH_3), 4.11–4.42 (m, 4H, CH_2CH_3), 1.54–1.61 (m, 6H, CH_3CH_2). ^{13}C NMR: (DMSO-d_6) δ: 175.18 (C), 164.97 (C), 148.37 (CH), 141.22 (C), 134.31 (C), 129.93 (C), 127.66 (C), 120.88 (C), 109.82 (C), 107.83 (CH), 62.06 (CH_2), 46.39 (CH_3), 35.59 (CH_3), 23.38 (CH_2), 14.17 (CH_3), 14.59 (CH_3). GC/MS: 331 (M + H). Anal. Calcd for ($C_{16}H_{18}N_4O_4$): C, 58.17; H, 5.49; N, 16.96. Found: C, 58.20; H, 5.46; N, 16.99.

*5.1.12. General procedure for the synthesis of alkoxy-dimethyl-6-oxo-6,9-dihydro-triazoloquinoline-carboxylic acid derivatives **21a,c** and **30a***

0.50 g of the appropriate triazolo[4,5-h]quinoline-carboxylate (**20a,c** or **29a**) are dissolved in 20 ml of NaOH 2 M. The reaction temperature is brought to 100 °C and allowed to react for one hour. Returned to room temperature, the solution is acidified with HCl 6 M, obtaining complete precipitation. The gelatinous solid is filtered, washed with plenty of water, and dried, obtaining the pure product.

5.1.12.1. 4-Methoxy-3,9-dimethyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (21a). M.P.> 300 °C. Yield: 89%. ^{1}H NMR: (DMSO-d_6) δ: 9.04 (s, 1H, H8), 7.68 (s, 1H, H5), 4.66 (s, 3H, O-CH_3), 4.54 (s, 3H, N3-CH_3), 4.14 (s, 3H, N_9-CH_3). ^{13}C NMR: (DMSO-d_6) δ: 175.16 (C), 166.23 (C), 150.98 (CH), 143.88 (C), 136.91 (C), 133.57 (C), 130.41 (C), 127.82 (C), 109.57 (C), 103.66 (CH), 55.89 (CH_3), 47.17 (CH_3), 35.22 (CH_3). GC/MS: 289 (M + H). Anal. Calcd for ($C_{13}H_{12}N_4O_4$): C, 54.17; H, 4.20; N, 19.44. Found: C, 54.15; H, 4.22; N, 19.46.

*5.1.12.2. 4,6-Dimethoxy-1-methyl-1H- [1–3]triazolo[4,5-h]quinoline-7- carboxylic acid (**21c**).* M.P.> 300 °C. Yield: 42%. ^{1}H NMR: ($CDCl_3$) δ: 8.89 (s, 1H, H8), 7.83 (s, 1H, H5), 4.61 (s, 3H, O-CH_3), 4.42 (s, 3H, N3-CH_3), 4.01 (s, 3H, -CH_3). ^{13}C NMR: (DMSO-d_6) δ: 175.77 (C), 165.95 (C), 147.27 (CH), 144.96 (C), 138.12 (C), 129.48 (C), 127.66 (C), 123.63 (C), 109.22 (C), 100.66 (CH), 59.87 (CH_2), 56.77 (CH_3), 52.63 (CH_2), 37.39 (CH_3), 15.73 (CH_3). GC/MS: 288 (M + H). Anal. Calcd for ($C_{13}H_{12}N_4O_4$): C, 54.17; H, 4.20; N, 19.44. Found: C, 54.12; H, 4.23; N, 19.42.

*5.1.12.3. 4-Ethoxy-3,9-dimethyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**30a**).* M.P.> 300 °C. Yield: 82%. ^{1}H NMR: ($CDCl_3$) δ: 15.62 (s, 1H, OH), 8.90 (s, 1H, H8), 7.73 (s, 1H, H5), 4.73 (s, 3H, CH_3), 4.61 (s, 3H, OCH_3), 4.40 (Q, 2H, CH_2CH_3), 1.61 (t, 3H, CH_3CH_2). ^{13}C NMR: (DMSO-d_6) δ: 176.39 (C), 165.74 (C), 151.66 (CH), 140.23 (C), 136.17 (C), 133.98 (C), 130.52 (C), 127.82 (C), 110.32 (C), 102.84 (CH), 64.31 (CH_2), 46.37 (CH_3), 35.89 (CH_3), 15.03 (CH_3). GC/MS: 303 (M + H). Anal. Calcd for ($C_{14}H_{14}N_4O_4$): C, 55.63; H, 4.67; N, 18.53. Found: C, 55.66; H, 4.70; N, 18.50.

*5.1.13. General procedure for the synthesis of methoxy-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylate derivatives (**22a-c, 31a**)*

0.5 g of the adequate ethyl-alkoxy-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylate derivatives (**19a-c** or **28a**) are added to a suspension of 0.35 g of KOH in 15 ml of DMSO at room temperature. After 30 min, 0.55 ml of CH_3CH_2I are added. After 30 min from the addition an abundant precipitate is formed. The stirring is removed and the solution is diluted with 40 ml of water, obtaining a solid which is filtered and dried.

*5.1.13.1. Ethyl 9-ethyl-4-methoxy-3-methyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**22a**).* M.P.: 225–226 °C. Yield: 90%. ^{1}H NMR: (DMSO-d_6) δ: 8.63 (s, 1H, H8), 7.59 (s, 1H, H5), 5.02 (q, 2H, O- CH_2-), 4.48 (s, 3H, NCH_3), 4,26 (q, 2H, N-CH_2-),4.04 (s, 3H, OCH_3), 1.42 (t, 3H, CH_3 etile), 1.32 (t, 3H, CH_3). ^{13}C NMR: (DMSO-d_6) δ: 171.18 (C), 164.60 (C), 147.25 (CH), 143.58 (C), 138.37 (C), 128.67 (C), 126.34 (C), 126.05 (C), 111.80 (C), 102.05 (CH), 59.87 (CH_2), 56.41 (CH_3), 51.34 (CH_2), 37.17 (CH_3), 15.76 (CH_3), 14.26 (CH_3). GC/MS: 331 (M + H). Anal. Calcd for ($C_{16}H_{18}N_4O_4$): C, 58.17; H, 5.49; N, 16.96. Found: C, 58.19; H, 5.46; N, 16.99.

*5.1.13.2. Ethyl 9-ethyl-4-methoxy-2-methyl-6-oxo-6,9-dihydro-2H-[1,2,3]triazolo[4,5-**h**]quinoline-7-carboxylate (**22b**).* M.P.: 239–240 °C. Yield: 66%. ^{1}H NMR: (DMSO-d_6) δ: 8.64 (s, 1H, H8), 7.52 (s, 1H, H5), 4.90 (q, 2H, CH_2CH_3), 4.58 (s, 3H, OCH_3), 4.25 (q, 2H, CH_2CH_3), 4.03 (s, 3H, CH_3), 1.43 (t, 3H, CH_3CH_2), 1.31 (t, 3H, CH_3CH_2). ^{13}C NMR: (DMSO-d_6) δ: 171.27 (C), 164.60 (C), 147.10 (C), 146.63 (CH), 139.66 (C), 136.91 (C), 127.40 (C), 126.11 (C), 112.20 (C), 99.38 (CH), 59.89 (CH_2), 55.88 (CH_3), 51.02 (CH_2), 40.42 (CH_3), 15.80 (CH_3), 14.25 (CH_3). GC/MS: 331 (M + H). Anal. Calcd for ($C_{16}H_{18}N_4O_4$): C, 58.17; H, 5.49; N, 16.96. Found: C, 58.15; H, 5.52; N, 16.93.

*5.1.13.3. Ethyl 6-ethoxy-4-methoxy-1-methyl-1H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**22c**).* M.P.: 105–106 °C. Yield: 84%. ^{1}H NMR: (DMSO-d_6) δ: 8.97 (s, 1H, H8), 7.16 (s, 1H, H5), 4.71 (s, 3H, CH_3), 4.42 (q, 2H, CH_2CH_3), 4.27 (q, 2H, CH_2CH_3), 4.11 (s, 3H, CH_3), 1.47 (t, 3H, CH_3CH_2), 1.39 (t, 3H, CH_3CH_2). ^{13}C NMR: (DMSO-d_6) δ: 164.60 (C), 161.34 (C), 149.74 (C), 148.39 (CH), 140.02 (C), 136.38 (C), 130.47 (C), 123.74 (C), 116.46 (C), 94.60 (CH), 71.82 (CH_2), 61.48 (CH_2), 56.01 (CH_3), 37.57 (CH_3), 15.33 (CH_3), 13.96 (CH_3). GC/MS: 331 (M + H). Anal. Calcd for ($C_{16}H_{18}N_4O_4$): C, 58.17; H, 5.49; N, 16.96. Found: C, 58.16; H, 5.50; N, 16.94.

*5.1.13.4. Ethyl 4-ethoxy-9-ethyl-3-methyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**31a**).* M.P.: 192–193 °C. Yield: 73%. ^{1}H NMR: ($CDCl_3$) δ: 8.49 (s, 1H, H8), 7.88 (s, 1H, H5), 5.10 (q, 2H, N- CH_2CH_3), 4.56 (s, 3H, CH_3), 4.28–4.48 (m, 4H, 2 CH_2CH_3), 1.41–1.67 (m, 9H, 3 CH_3CH_2). ^{13}C NMR: (DMSO-d_6) δ: 174.32 (C), 164.09 (C), 146.20 (CH), 142.98 (C), 138.66 (C), 128.03 (C), 126.49 (C), 126.05 (C), 110.83 (C), 102.57 (CH), 64.31 (CH_2), 61.40 (CH_2), 51.86 (CH_2), 36.22 (CH_3), 14.41 (CH_3), 14.32 (CH_3), 14.26 (CH_3). GC/MS: 344 (M + H).). Anal. Calcd for ($C_{17}H_{20}N_4O_4$): C, 59.29; H, 5.85; N, 16.27. Found: C, 59.31; H, 5.88; N, 16.26.

*5.1.14. General procedure for the synthesis of alkoxy-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylic acid (**23a-c, 32a**)*

0.5 g of the adequate alkoxy-methyl-6-oxo-6,9-dihydro-triazolo [4,5-h]quinoline-7-carboxylate derivatives (**22a-c** or **31a**) are dissolved in 22 ml of 2 M NaOH. The mixture is brought to 100 °C for 2 h under magnetic stirring. The basic, clear solution is returned to room temperature and subsequently acidified with 2 M HCl; a very fine gelatinous precipitate is formed which is filtered (albeit with difficulty) and dried, obtaining pure carboxylic acid.

*5.1.14.1. 9-Ethyl-4-methoxy-3-methyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**23a**).* M.P.> 300 °C. Yield: 86%. ^{1}H NMR: (DMSO-d_6) δ: 15.11 (s, 1H, OH), 9.08 (s, 1H, H8), 7.68 (s, 1H, H5), 5.25 (q, 2H, CH_2-CH_3), 4,54 (s, 3H, NCH_3), 4.13 (s, 3H, OCH_3), 1.50 (t, 3H, CH_3-CH_2). ^{13}C NMR: (DMSO-d_6) δ: 175.77 (C), 165.95 (C), 147.27 (CH), 144.96 (C), 138.12 (C), 129.48 (C), 127.66 (C), 123.63 (C), 109.22 (C), 100.66 (CH), 59.87 (CH_2), 56.77 (CH_3), 52.63 (CH_2), 37.39 (CH_3), 15.73 (CH_3). GC/MS: 303 (M + H). Anal. Calcd for ($C_{14}H_{14}N_4O_4$): C, 55.63; H, 4.67; N, 18.53. Found: C, 55.60; H, 4.64; N, 18.54.

*5.1.14.2. 9-Ethyl-4-methoxy-2-methyl-6-oxo-6,9-dihydro-2H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**23b**).* M.P.: 273–274 °C. Yield: 95%. ^{1}H NMR: (DMSO-d_6) δ: 15.83 (s, 1H, OH), 9.03 (s, 1H, H8), 7.47 (s, 1H, H5), 5.05 (m, 2H, CH_2CH_3), 4.62 (s, 3H, OCH_3), 4.08 (s, 3H, CH_3), 1.47 (t, 3H, CH_3CH_2). ^{13}C NMR: (DMSO-d_6) δ: 175.71 (C), 166.00 (C), 148.31 (C), 146.46 (CH), 142.27 (C), 140.14 (C), 127.56 (C), 125.03 (C), 111.10 (C), 99.05 (CH), 56.18 (CH_3), 52.30 (CH_2), 43.99 (CH_3), 15.69 (CH_3). GC/MS: 303 (M + H). Anal. Calcd for ($C_{14}H_{14}N_4O_4$): C, 55.63; H, 4.67; N, 18.53. Found: C, 55.66; H, 4.70; N, 18.51.

*5.1.14.3. 6-Ethoxy-4-methoxy-1-methyl-1H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**23c**).* M.P.: 194–195 °C. Yield: 76%. ^{1}H NMR: (DMSO-d_6) δ: 8.98 (s, 1H, H8), 7.17 (s, 1H, H5), 4.71 (s, 3H, OCH_3), 4.32 (q, 2H, CH_2), 4.11 (s, 3H, CH_3), 1.46 (t, 3H, CH_3). ^{13}C NMR: (DMSO-d_6) δ: 166.16 (C), 161.25 (C), 149.58 (C), 148.66 (CH), 139.90 (C), 136.19 (C), 130.45 (C), 123.78 (C), 117.25 (C), 94.58 (CH), 71.55 (CH_2), 57.21 (CH_3), 37.55 (CH_3), 15.57 (CH_3). GC/MS: 303 (M + H). Anal. Calcd for ($C_{14}H_{14}N_4O_4$): C, 55.63; H, 4.67; N, 18.53. Found: C, 55.62; H, 4.68; N, 18.55.

*5.1.14.4. 4-Ethoxy-9-ethyl-3-methyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**32a**).* M.P.: 263–264 °C. Yield: 57%. ^{1}H NMR: ($CDCl_3$) δ: 15.43 (s, 1H, OH), 8.75 (s, 1H, H8), 7.75 (s, 1H, H5), 5.21 (q, 2H, OCH_2CH_3), 4.40 (q, 2H, OCH_2CH_3), 1.60 (m, 6H, 2 CH_3CH_2). ^{13}C NMR: (DMSO-d_6) δ: 175.29 (C), 166.37 (C), 149.09 (CH), 140.33 (C), 138.01 (C), 128.47 (C), 126.39 (C), 126.37 (C), 110.77 (C), 102.67 (CH), 64.11 (CH_2), 51.32 (CH_2), 35.41 (CH_3), 14.87 (CH_3), 14.26 (CH_3). GC/MS: 316 (M + H). Anal. Calcd for ($C_{15}H_{16}N_4O_4$): C, 56.96; H, 5.10; N, 17.71. Found: C, 56.99; H, 5.08; N, 17.74.

*5.1.15. General procedure for the synthesis of 4-methoxy-1/3-methyl-6-oxo-6,9-dihydro-1H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**24a,c**)*

0.5 g of the adequate mehoxy-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinoline-7-carboxylate derivatives (**19a,c**) are dissolved in 22 ml of 2 M NaOH. The mixture is brought to 100 °C for 2 h under magnetic stirring. The basic, clear solution is returned to room temperature and subsequently acidified with 2 M HCl; a precipitate is formed which is filtered and dried, obtaining pure carboxylic acid.

*5.1.15.1. 4-Methoxy-3-methyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**24a**).* M.P.> 300 °C. Yield: 74%. ^{1}H NMR: ($CDCl_3$) δ: 8.615 (s, 1H, H8), 7.507 (s, 1H, H5), 4.511 (s, 3H, N-CH_3), 4.109 (s, 3H, O-CH_3). ^{13}C NMR: (DMSO-d_6) δ: 174.88 (C), 165.79 (C), 150.23 (CH), 144.21 (C), 139.97 (C), 132.09 (C), 130.56 (C), 127.37 (C), 111.68 (C), 103.17 (CH), 55.77 (CH_3), 35.43 (CH_3). GC/MS: 274 (M + H). Anal. Calcd for ($C_{12}H_{10}N_4O_4$): C, 52.56; H, 3.68; N, 20.43. Found: C, 52.54; H, 3.71; N, 20.39.

*5.1.15.2. 4-Methoxy-1-methyl-6-oxo-6,9-dihydro-1H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**24c**).* M.P.> 300 °C. Yield: 97%. ^{1}H NMR: ($CDCl_3$) δ: 8.747 (s, 1H, H8), 7.514 (s, 1H, H5), 4.695 (s, 3H, N-CH_3), 3.951 (s, 3H, O-CH_3). ^{13}C NMR: (DMSO-d_6) δ: 175.31 (C), 165.06 (C), 150.45 (CH), 144.19 (C), 140.22 (C), 132.35 (C), 130.25 (C), 127.56 (C), 110.41 (C), 102.89 (CH), 55.37 (CH_3), 35.36 (CH_3). GC/MS: 274 (M + H). Anal. Calcd for ($C_{12}H_{10}N_4O_4$): C, 52.56; H, 3.68; N, 20.43. Found: C, 52.58; H, 3.66; N, 20.41.

*5.1.16. Synthesis of ethylthio-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h]quinolines (**33**–**37**)*

*5.1.16.1. 7-(ethylthio)-1-methyl-4-nitro-1H-benzo[d][1,2,3]triazole (**33**).* 1 g (5.1×10^{-3} mol) of benzotriazole intermediate **8a** are dissolved in 5 ml (6×10^{-2} mol) 3.7 g of thioethane with 2 ml of Et_3N. The solution is refluxed for 4 h, then brought to room temperature. After evaporation, a pale yellow solid is collected. The raw compound is purified through flash chromatography.). M.P.: 193–194 °C. Yield: 76%. ^{1}H NMR: ($CDCl_3$) δ: 8.17 (d, 1H, $J = 8.4$ Hz, H6), 7.23 (d, 1H, $J = 8.4$ Hz, H5), 4.67 (s, 3H, CH_3), 3.23 (q, 2H, CH_2), 1.50 (t, 3H, CH_3). GC/MS: 239 (M + H). Anal. Calcd for ($C_9H_{10}N_4O_2S$): C, 45.37; H, 4.23; N, 23.51; S, 13.46. Found C, 45.40; H, 4.20; N, 23.53; S, 13.49.

*5.1.16.2. 7-(ethylthio)-1-methyl-1H-benzo[d][1,2,3]triazol-4-amine (**34**).* 1.0 g ($4.2 \cdot 10^{-3}$ mol) of 7-(ethylthio)-1-methyl-4-nitro-1*H*-benzotriazole (**35**) are dissolved in 50 ml of ethanol and added with 0.10 g of 10% C/Pd. It is set to reduce by means of a hydrogenator; the theoretical amount of H_2 is rapidly absorbed at room temperature and at atmospheric pressure. By filtration the catalyst is removed, while the solvent is removed by evaporation. The pure amine is obtained as a violet oil. Oil. Yield: 84%. ^{1}H NMR ($CDCl_3$) δ: 7.36 (d, 2H, J = 7.8, H6), 6.42 (d, 2H, J = 7.8, H5), 4.58 (s, 3H, CH_3), 2.72 (q, 2H, CH_2), 1.19 (t, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 146.9 (C), 140.7 (C), 133.2 (C), 130.5 (C), 126.8 (CH), 121.1 (CH), 33.2 (CH_3), 28.8 (CH_2), 14.1 (CH_3). GC/MS: 209 (M + H). Anal. Calcd for ($C_9H_{12}N_4S$): C, 51.90; H, 5.81; N, 26.90; S, 15.39. Found: C, 51.92; H, 5.79; N, 26.93; S, 15.41.

*5.1.16.3. Ethyl 4-(ethylthio)-3-methyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**35**).* 1 g ($5.2 \cdot 10^{-3}$ mol) of 7-(ethylthio)-1-methyl-1H-benzotriazol-4-amine (**34**) is suspended in 25 ml of Dowtherm, then 1.8 g (8.6×10^{-3} mol) 1.7 ml of diethyl ethoxymethylenemalonate (EMME) are added. The solution is brought to 150 °C for 17 h, but reaction resulted incomplete. We brought temperature to 250 °C and we let it go for almost 30 min. Finally, we reported the reaction temperature at room temperature, and we added 30 ml of hexane to obtain an abundant precipitate. The solid compound is filtered, washed with firstly with hexane, then with diethyl ether and dried. Raw precipitate is purified through flash chromatography obtaining pure quinolone derivative. M.P.: 225–226 °C. Yield: 66%. ^{1}H NMR ($CDCl_3$) δ: 12.85 (s, 1 H, NH), 8.82 (s, 1H, H-5), 8.03 (s, 1H, H-8), 4.61 (s, 3H, N-CH_3), 4.47 (q, 2H, O-CH_2, J = 7.0), 3.10 (q, 2H, S-CH_2, J = 7.2), 1.51–1.37 (m, 6H, 2 CH_3). ^{13}C NMR: (DMSO-d_6) δ: 174.33 (C), 165.42 (C), 149.11 (CH), 141.64 (C), 134.25 (C), 129.34 (C), 126.89 (C), 121.34 (C), 111.02 (C), 115.22 (CH), 61.81 (CH_2), 35.12 (CH_3), 29.57 (CH_2), 14.45 (CH_3), 14.29 (CH_3). GC/MS: 333 (M + H). Anal. Calcd for (C15H16N4O3S): C, 54.20; H, 4.85; N, 16.86; S, 9.65. Found: C, 54.22; H, 4.83; N, 16.89; S, 9.63.

*5.1.16.4. Ethyl 4-(ethylthio)-3,9-dimethyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**36**).* 1 g ($3.0 \cdot 10^{-3}$ mol) of triazolo-quinoline-7-carboxylate (**35**) are added to a suspension of 0.48 g of KOH in 20 ml of DMSO at room temperature. After 20 min, 0.5 ml ($8 \times 10_{-3}$ mol), 1.15 g of CH_3I are added and the reaction is left for 4 h. The dark red solution is then diluted with 25 ml of water, obtaining a brown solid, which is filtered and dried. We get an impure solid which is purified through flash chromatography. M.P.: 214–215 °C. Yield: 83%. ^{1}H NMR ($CDCl_3$) δ: 9.08 (s, 1 H, H-5), 8.14 (s, 1H, H-8), 4.73 (s, 3H, N-CH_3), 4.70 (s, 3H, N-CH_3), 4.24 (q, 2H, O-CH_2, J = 7.0), 3.23 (q, 2H, S-CH_2, J = 7.2), 1.61–1.25 (m, 6H, 2 CH_3). ^{13}C NMR: (DMSO-d_6) δ: 174.87 (C), 165.17 (C), 149.83 (CH), 141.01 (C), 134.52 (C), 129.39 (C), 127.77 (C), 121.17 (C), 111.25 (C), 115.32 (CH), 61.69 (CH_2), 46.21 (CH_3), 35.12 (CH_3), 29.56 (CH_2), 14.32 (CH_3), 14.21 (CH_3). GC/MS: 347 (M + H). Anal. Calcd for ($C_{16}H_{18}N_4O_3S$): C, 55.48; H, 5.24; N, 16.17; S, 9.26. Found: C, 55.50; H, 5.22; N, 16.19; S, 9.23.

*5.1.16.5. 4-(ethylthio)-3,9-dimethyl-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**37**).* 0.5 g (1.5×10^{-3} mol) of triazolo-quinoline-carboxylate (**36**) are dissolved in 22 ml of 2 M NaOH. The mixture is brought to 100 °C for 2 h under magnetic stirring. After cooling, the solution is neutralized with 2 M HCl; a very fine gelatinous precipitate is formed, which is filtered and dried giving the pure product. M.P.> 300 °C. Yield: 73%. ^{1}H NMR ($CDCl_3$) δ: 15.12 (s, 1H, OH) 8.76 (s, 1 H, H-5), 8.30 (s, 1H, H-8), 4.76 (s, 3H, N-CH_3), 4.68 (s, 3H, N-CH_3), 3.27 (q, 2H, S-CH_2, J = 7.2), 1.50–1.43 (m, 3H, CH_3). ^{13}C NMR: (DMSO-d_6) δ: 174.68 (C), 165.32 (C), 149.56 (CH), 141.39 (C), 134.27 (C), 129.81 (C), 127.83 (C), 121.143 (C), 111.46 (C), 115.07 (CH), 45.79 (CH_3), 35.26 (CH_3), 29.43 (CH_2), 14.39 (CH_3). GC/MS: 319 (M + H). Anal. Calcd for ($C_{14}H_{14}N_4O_3S$): C, 52.82; H, 4.43; N, 17.60; S, 10.07. Found: C, 52.79; H, 4.45; N, 17.58; S, 10.10.

*5.1.17. Synthesis of nitro-methyl-6-oxo-6,9-dihydro-triazolo[4,5-h] quinolines (**38**–**41**)*

*5.1.17.1. 1-Methyl-7-nitro-1H-benzo[d][1,2,3]triazol-4-amine (**38**).* 1 g (5.09×10^{-2} mol) of 7-fluoro-1-methyl-4-nitro-1*H*-benzo[*d*][1,2,3]triazole (**8c**) are dissolved in 100 ml of ammonia propanol and placed in a closed tube at 50 °C for 2 h. The abundant precipitate obtained is cooled and filtered, which is washed with ethyl ether and dried as a pure compound. M.P. 256–257 °C. Yield: 98%. ^{1}H NMR ($CDCl_3$) δ: 8.18 (d, 1H, *J* = 8.8 Hz, H6), 6.46 (d, 1H, J = 8.8, H-6), 4.47 (s, 3H, CH_3). GC/MS: 194 (M + H). Anal. Calcd for ($C_7H_7N_5O_2$): C, 43.53; H, 3.65; N, 36.26. Found: C, 43.50; H, 3.67; N, 36.23.

*5.1.17.2. Diethyl 2-(((1-methyl-7-nitro-1H-benzo[d][1,2,3]triazol-4-yl)amino)methylene)malonate (**39**).* 0.8 g (4.3×10^{-3} mol) of 1-methyl-7-nitro-1*H*-benzo[*d*][1,2,3]triazol-4-amine (**38**) are suspended in 15 ml of Dowtherm. 1.03 g (4.73×10^{-3} mol) 0.96 ml of diethyl ethoxymethylenemalonate (EMME) are added. The solution is brought to 150 °C and let go for 17 h. Once the time has expired, the mixture is brought to room temperature. Hexane is then added to obtain a precipitate which is filtered, washed with hexane and dried. Pure compound is obtained through flash chromatography. M.P.: 159–160 °C. Yield: 75%. ^{1}H NMR ($CDCl_3$) δ: 11.973 (d, 1H, *J* = 11.8 Hz, NH), 9.064 (d, 1H, =CH, J = 12), 8.405 (d, 1H, H6, *J* = 9 Hz), 7.076 (d, 1H, H5, *J* = 9 Hz), 4.655 (s, 3H, CH_3), 4.463–4.263 (m, 4H, 2 CH_2), 1.461–1.346 (m, 6H, 2 CH_3). GC/MS: 364 (M + H). Anal. Calcd for ($C_{15}H_{17}N_5O_6$): C, 49.59; H, 4.72; N, 19.28. Found: C, 49.62; H, 4.74; N, 19.25.

*5.1.17.3. Ethyl 3-methyl-4-nitro-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**40**).* 20 ml of Dowtherm are brought to the boiling temperature, then 0.7 g (1.9×10^{-3} mol) of diethyl 2-(((1-methyl-7-nitro-1*H*-benzo[*d*][1,2,3]triazol-4-yl) amino)methylene)malonate (**39**) are then rapidly added. After 35 min, the solution is allowed to cool down. A very fine precipitate is observed, which is filtered and washed with hexane and diethyl ether, obtaining the pure compound. M.P.: 256–257 °C. Yield: 72%. ^{1}H NMR ($CDCl_3$) δ: 13.720 (s, 1H, NH), 8.873 (s, 1 H, H-5), 8.460 (s, 1H, H-8), 4.569 (s, 3H, CH_3), 4.262 (q, 2H, CH_2), 1.306 (t, 3H, CH_3).). ^{13}C NMR: ($CDCl_3$) δ: 174.19 (C), 165.16 (C), 153.67 (C), 146.46 (CH), 142.71 (C), 134.47 (C), 130.21 (C), 129.45 (C), 112.29 (C), 112.06 (CH), 61.45 (CH_2), 34.69 (CH_3), 14.33 (CH_3). GC/MS: 318 (M + H). Anal. Calcd for ($C_{13}H_{11}N_5O_5$): C, 49.22; H, 3.49; N, 22.07. Found: C, 49.19; H, 3.51; N, 22.10.

*5.1.17.4. 3,9-Dimethyl-4-nitro-6-oxo-6,9-dihydro-3H-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylic acid (**41**).* 1.05 g (3.3×10^{-3}) of ethyl 3-methyl-4-nitro-6-oxo-6,9-dihydro-3*H*-[1,2,3]triazolo[4,5-h]quinoline-7-carboxylate (**40**) are added to a suspension of 0.73 g of KOH in 15 ml of DMSO at room temperature. After 30 min, 0.85 ml of CH_3I are added. After 20 min, an abundant precipitate appears which is filtered and washed with chloroform. M.P.> 300 °C. Yield: 30%. ^{1}H NMR ($CDCl_3$) δ: 9.556 (s, 1 H, H5), 8.235 (s, 1H, H8), 4.309 (s, 3H, CH_3), 3.55 (s, 3H, CH_3). ^{13}C NMR: (DMSO-d_6) δ: 174.03 (C), 164.21 (C), 152.12 (C), 151.36 (CH), 143.66 (C), 135.26 (C), 131.79 (C), 130.22 (C), 112.90 (C), 112.37 (CH), 45.29 (CH_3), 35.07 (CH_3). GC/MS: 304 (M + H). Anal. Calcd for ($C_{12}H_9N_5O_5$): C, 47.53; H, 2.99; N, 23.10. Found: C, 47.50; H, 3.02; N, 23.13.

5.2. Anti-mycobacterial activity assay

5.2.1. Resazurin Microtiter Assay (REMA)

100 μl 7H9 was dispensed in each well of a sterile 96-well plate, and serial twofold dilutions of essential oil were prepared directly on the plate by adding 100 μl of the working solution of drug to achieve the final concentration. For all mycobacteria strains the FBT concentration range used was 32−1 μg/ml. The inoculum was prepared from the 7H9 growth, adjusted to a McFarland tube scale 1. The suspension was diluted 1:10 and 100 μl was added to each well. The plates were covered, sealed in plastic bags, and incubated for 7 days at 37 °C. After the final visual reading, 30 μl of 0.02% resazurin was added to each well and re-incubated overnight. A change in color from blue (oxidized state) to pink (reduced) indicated bacterial growth and Minimal inhibitory concentration (MIC) was defined as the lowest drug concentration that prevented the color change.

5.2.2. In vitro cytotoxicity assay

All compounds were tested for toxicity (EC_{50}) in a mammalian VERO cell line (ATCC CCL-81, ATCC, Manassas, VA, USA) at a concentration of 100 μM. The same concentration of ciprofloxacin was used for comparison. After 72 h of exposure, cell viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 non-radioactive cell proliferation assay (Promega, Madison, WI, USA).

5.2.3. Mycobacterium tuberculosis DNA gyrase inhibition assay

M. tuberculosis DNA gyrase supercoiling assays were performed in the absence or presence of compounds (**21a**), (**30a**), (**24a**), and ciprofloxacin as control using the *M. tuberculosis* Gyrase Supercoing Assay Kit according to the manufacturer's recommendations (Inspiralis, Norwick, UK). In brief, the assay (30 μL) was carried out in gyrase buffer (50 mM HEPES, KOH (pH = 7.9), 6 mM magnesium acetate 4 mM DTT, 1 mM ATP, 2 mM spermidine, 100 mM potassium glutamate, and 0.05 mg/mL of albumin) containing 0.5 μg of relaxed plasmid pBR322 DNA, using 1 U of *M. tuberculosis* DNA gyrase x assay buffer. Reactions were also carried in the presence of ciprofloxacin and test compounds (2 μL); their vehicles, water and DMSO served as negative control, respectively. Reactions were incubated for a period of 30 min at 37 °C, and subsequently quenched by the addition of 30 μL of STEB stop buffer (40% w/v sucrose, 100 mM Tris-HCl (pH 8), 10 mM EDTA, 0.5 mg/mL Bromophenol Blue) and 30 μL of 24:1 chloroform:isoamyl alcohol. Reaction products were then loaded onto 1% agarose TEA gels and allow to rest for 40 min prior to electrophoresis. Gels were then stained with 0.5 μg/l ethidium bromide, and images were analyzed using densitometry (ImageJ, NIH). The IC_{50} values for each tested compound were determined to be the compound concentration that inhibited *M. tuberculosis* DNA gyrase activity by 50% (determined fro densitometry values relative to positive and negative controls of reactions with or without the enzyme, respectively).

5.3. Antibacterial and anti-yeast activity assay

The strains used were form American Type Culture Colletion (ATCC): *S. aureus* ATCC 2913, *S. typhimurium* ATCC 14028, *E. faecalis* ATCC 2491 and *E. coli* ATCC 15922, and yeasts (*C. albicans* and *C. tropicalis*). The minimum inhibitory concentration (MIC) was determined according to the dilution method in broth with test tubes. Each compound was dissolved in dimethyl sulfoxide (DMSO), then diluted in Lb broth (Luria broth, Difco). The range of concentration used for each compound was 500−0.5 μg/ml. The final concentration of the inoculum was 10^6 CFU/mL. After overnight incubation at 37 °C, test organisms were diluted to the optical density of a 0.5 McFarland turbidity standard and measured at 450 nm. The MIC was determined as the lowest concentration of compound that completely inhibited bacteria growth.

5.4. In silico pharmacokinetic property predictions

All compounds were further subjected to *in silico* pharmacokinetic prediction using the online drug-likeness and molecular property prediction software (MolSoft L.L.C.; http://molsoft.com/mprop/). Ciprofloxacin was taken as the reference standard for comparing the compound series.

5.5. Molecular modeling

All simulations were performed with the AMBER 17 suite of programs [29]. The 3D model structure of the Mtb DNA gyrase was taken from the Protein Data Bank (file 5BTC.pdb [23]) Compound **21a** was then docked into the enzyme-binding site using Autodock 4.2 [30]. The resulting complex was further energy minimized to convergence. The intermolecular complex was then solvated and energy minimized using a combination of molecular dynamics (MD) techniques [25−27]. 20 ns molecular dynamics (MD) simulations at 298 K were then employed for system equilibration, and further, 50-ns MD were run for data production. Following the MM/PBSA approach [31] the affinity of Mtb DNA gyrase and 21a (ΔG_{bind}) was calculated as the sum of the electrostatic, van der Waals, polar solvation, nonpolar solvation, and entropic contributions. For validation and comparison purposes, the same procedure was applied also to the ciprofloxacin/enzyme complex.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2018.10.031.

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