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[1,2,3]Triazolo[4,5-*h*]quinolones. A new class of potent antitubercular agents against multidrug resistant *Mycobacterium tuberculosis* strains

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Abstract—In this preliminary study we report the activity of 3-methyl-9-substituted-6-oxo-6,9-dihydro-3*H*-[1,2,3]-triazolo [4,5-*h*]quinolone-carboxylic acids and their esters as a new class of antiinfective agents against MDR *Mycobacterium tuberculosis*. In antitubercular screening against H37Rv and 11 clinically isolated strains of *M. tuberculosis* several derivatives (**1o**,**3a**,**c**,**i**,**j**,**p**) showed MIC_{90} in the range 0.5–3.2 μg/mL. **3c** showed no cytotoxicity and proved to be the most potent derivative exhibiting MIC_{90} = 0.5 μg/mL against all *M. tuberculosis* strains and infected human macrophages (J774-A1) tested.

Mycobacterium tuberculosis (*M. tuberculosis*) is the etiological agent for the Tuberculosis (TB). The incidence of TB has steadily risen in the last years and TB is the world's second most common cause of death from infectious diseases, after acquired immunodeficiency syndrome (AIDS).[1] Furthermore, association of TB and AIDS is wide-spread.[2,3] This resurgence affects both developed and developing countries with high rates of human immunodeficiency virus (HIV) infection. The World Health Organization (WHO) has estimated that 8 million of people develop active TB every year and almost one-forth of these die.[4]

Prior to the advent of effective chemotherapy, 50% of patients with active pulmonary TB died within 2 years.[5] Since 1940s the introduction of the combination of Streptomycin and para-aminosalicylic acid in therapy[6] and successively, the addition of Isoniazid, Ethambutol, Rifampin and Pyrazinamide, generally used in combination, made possible a high decrease in the mortality and in the duration of treatment. Today the reemergence of TB infection is further complicated by an increase of *M. tuberculosis* strains resistant to drugs used in conventional antitubercular therapy (XDR-TB) which are becoming a threat to public health worldwide. The emergence of XDR-TB shows that the development of novel mechanism-based antitubercular agents is necessary.[7]

It is known that multidrug resistant (MDR) bacilli to both Isoniazid and Rifampin are sensitive to some fluoroquinolones (Ciprofloxacin, Ofloxacin, Levofloxacin, etc.) which inhibit the topoisomerases II and IV as well as the DNA gyrases, essential enzymes to maintain the supercoils in bacterial DNA.[8] Consequently a huge effort has been made by scientists in order to discover new quinolone derivatives endowed with antitubercular activity.[9–12]

In the past some of us have reported that new classes of heterocycles are also endowed with antitubercular activity. In fact a certain number of quinoxaline 1,4-dioxides and benzotriazole derivatives were described as in vitro antitubercular agents at very low concentrations.[13–16] Now, owing to our continuous interest in this field we

Abbreviations: TB, tuberculosis; AIDS, acquired immunodeficiency syndrome; WHO, World Health Organization; MDR, multidrug resistant; MIC_{90}, inhibition constant; EMME, diethyl ethoxymethylenemalonate; Dowtherm A, mixture of biphenyl and diphenyl ether (26.5:73.5 ratio); SAR, structure activity relationships; XDR-TB, extensive drug resistance tuberculosis.

Keywords: Triazolo[4,5-*h*]quinolones; MDR *M. tuberculosis*; In vitro antitubercular activity.

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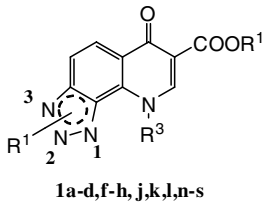
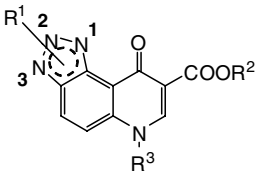
have taken into account a previously described class of angular triazolo[4,5-*h*] and [4,5-*f*]quinolone-carboxylic acids and their esters (**1a–d**,**f–h**,**j**,**k**,**l**,**n–s** and **2d–h**,**j**,**k–o** of Fig. 1) as antiinfectives of the urinary tract[17–19] as possible antitubercular agents on the basis that they are very closely related to fluoroquinolones but bear a triazole ring which can influence either the lipophilicity or the activity as whole. Furthermore, synthetic strategies and antibacterial activity of known triazoloquinolone derivatives have been reviewed by Milata.[20] In this context we have submitted those compounds to antitubercular test against *M. tuberculosis* H37Rv and thus we have observed that none of the triazolo[4,5-*f*]quinolone carboxylic derivatives were active, while instead three triazolo[4,5-*h*]quinolone carboxylic acids (**1c**,**n**,**o**,) exhibited interesting low MIC_{90} values (4.8, 5.0, and 1.6 μg/mL, respectively). In addition we have also observed that the activity was related to the length and position of the substituent at triazolo-nitrogen. In fact an ethyl group in **1s**, homologous of the most active **1o**, lowered its activity (MIC_{90} value from 1.6 to >32 μg/mL). Regarding the shift of the methyl group from nitrogen 3–2 or 1 position it was observed that the activity disappears. This observation prompted us to prepare the present new series of 3-methyl-9-substituted-6-oxo-6,9-dihydro-3*H*-[1,2,3]-triazolo[4,5-*h*]quinolone-carboxylic acids and their esters of structure **3a–p** depicted in Figure 2, where we considered a variety of substituents on the quinolone nitrogen in order to improve activity as such.

The general synthesis of **3a–p** is outlined in Scheme 1. The preparation of the known key intermediate (**9**)[19] was further modified to improve the yield. The commercially available benzotriazole (**4**) underwent nitration with a mixture of nitric acid (96%) and sulfuric acid (95%). A mixture of regioisomers (**5a**,**b**) in 3:1 ratio was obtained and recrystallization from acetone afforded **5a** in high yield (73%), avoiding tedious chromatography



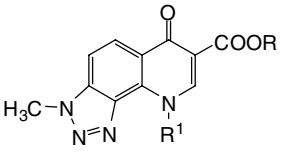
1a-d,f-h, j,k,l,n-s



2d-h,j,k-o

a) R^1=R^2=R^3=H;
b) R^1=N_2-CH_3, R^2=R^3=H;
c) R^1=N_3-CH_3, R^2=R^3=H;
d) R^1=R^3=H, R^2=CH_2CH_3;
e) R^1=N_1-CH_3, R^2=CH_2CH_3, R^3=H;
f) R^1=N_2-CH_3, R^2=CH_2CH_3, R^3=H;
g) R^1=N_3-CH_3, R^2=CH_2CH_3, R^3=H;
h) R^1=H, R^2=R^3=CH_2CH_3;
i) R^1=N_1-CH_3, R^2=R^3=CH_2CH_3;
j) R^1=N_2-CH_3, R^2=R^3=CH_2CH_3;
k) R^1=N_3-CH_3, R^2=R^3=CH_2CH_3;
l) R^1=R^2=H, R^3=CH_2CH_3;
m) R^1=N_1-CH_3, R^2=H, R^3=CH_2CH_3;
n) R^1=N_2-CH_3, R^2=H, R^3=CH_2CH_3;
o) R^1=N_3-CH_3, R^2=H, R^3=CH_2CH_3;
p) R^1=N_2-CH_2CH_3, R^2=R^3=CH_2CH_3;
q) R^1=N_3-CH_2CH_3, R^2=R^3=CH_2CH_3;
r) R^1=N_2-CH_2CH_3, R^2=H, R^3=CH_2CH_3;
s) R^1=N_3-CH_2CH_3, R^2=H, R^3=CH_2CH_3;

Figure 1. 6-Oxo-6,9-dihydro-3*H*-[1,2,3]-triazolo[4,5-*h*]quinoline-7-carboxylic acids and ethyl esters (**1a–d**,**f–h**,**j**,**k**,**l**,**n–s**) and 6-oxo-6,9-dihydro-3*H*-[1,2,3]-triazolo[4,5-*f*]quinoline-7- carboxylic acids and ethyl esters (**2d–h**,**j**,**k–o**) tested.



3a-p

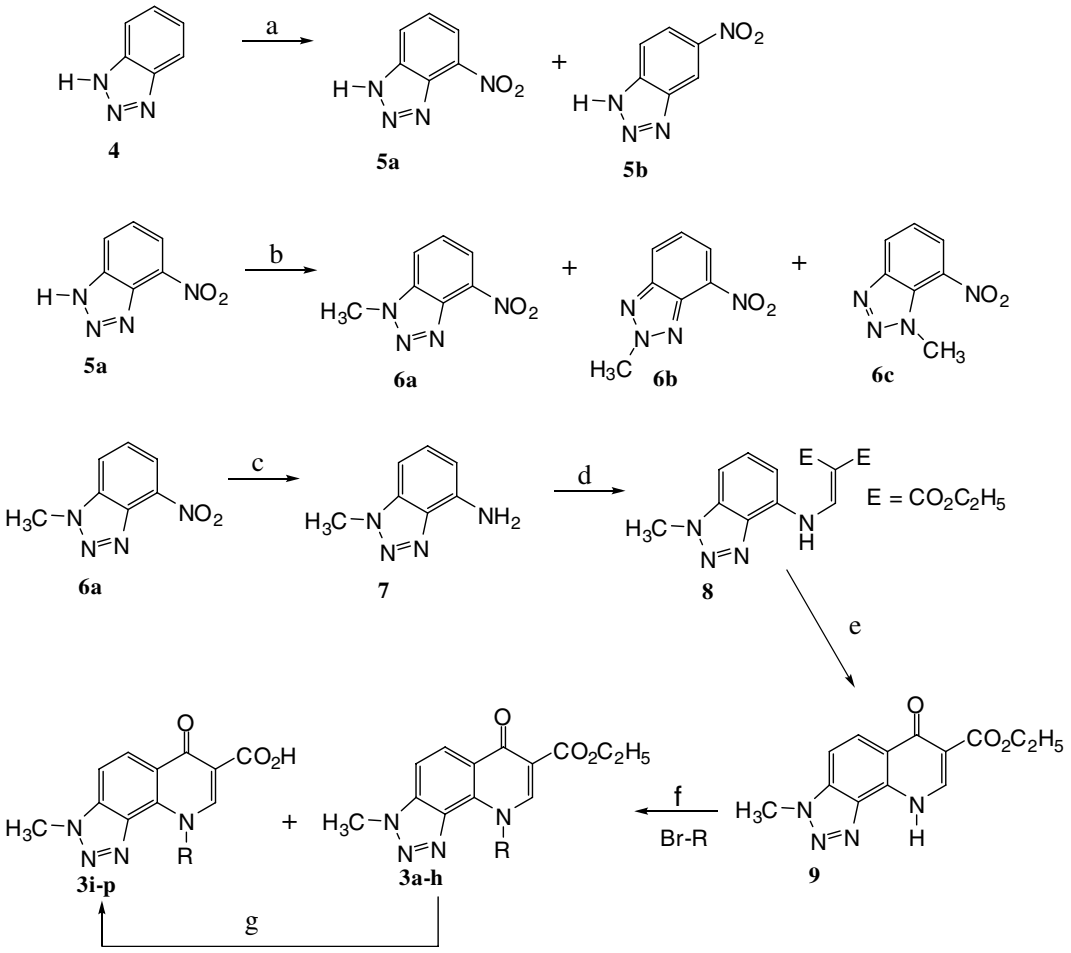
a) R=CH_2CH_3; R^1=CH_3;
b) R=CH_2CH_3; R^1=$CH_2CH_2CH_3$;
c)R=CH_2CH_3; R^1=$CH_2CH_2CH_2CH_3$;
d) R=CH_2CH_3; R^1=CH_2CH=CH_2;
e) R=CH_2CH_3; R^1=benzyl;
f) R=CH_2CH_3; R^1=4-Me-benzyl;
g) R=CH_2CH_3; R^1=4-Br-benzyl;
h) R=CH_2CH_3; R^1=CH_2CH_2-phenyl;
i) R=H; R^1=CH_3;
j) R=H; R^1=$CH_2CH_2CH_3$;
k) R=H; R^1=$CH_2CH_2CH_2CH_3$;
l) R=H; R^1=CH_2CH=CH_2;
m) R=H; R^1=benzyl;
n) R=H; R^1=4-Me-benzyl;
o) R=H; R^1=4-Br-benzyl;
p) R=H; R^1=CH_2CH_2-phenyl;

Figure 2. 3-Methyl-9-substituted-6-oxo-6,9-dihydro-3*H*-[1,2,3]-triazolo[4,5-*h*]quinoline-7-carboxylic acids and ethyl esters (**3a–p**).

processes. Alkylation with dimethyl sulfate of **5a** yielded the mixture of the three methyl isomers (**6a–c**) from which after chromatography resolution **6a** was obtained in 20% yield. The isomers (**5a**,**b**) and (**6a–c**) were unambiguously identified according to procedures previously reported.[21] Hydrogenation of **6a** under moderate pressure (Parr apparatus) afforded the aminoderivative (**7**) in quantitative yield, which according to Gould–Jacobs reaction with diethyl ethoxymethylenemalonate (EMME) in Dowtherm A gave the aminomethylenemalonate (**8**) in 96% yield. Thermal ring closure of **8** at 250 °C in Dowtherm A gave the corresponding quinolone **9** in 75% yield. The latter underwent alkylation at *N*-9 position with the appropriate bromide in the presence of sodium hydride to give the corresponding 9-substituted-triazolo[4,5-*h*]quinolone esters (**3a–h**) in 10–45% yields accompanied by their corresponding acids (**3i–p**) in 5–65%. All the acids (**3i–p**) were also obtained in 90–98% yields on alkaline hydrolysis of the esters (**3a–h**).

All the new derivatives (**3a–p**) were tested against H37Rv and further 11 clinically isolated strains of *M. tuberculosis* (SS1-11) endowed with different drug-resistance (Table 1). Comparison of the activity of our compounds with four standard antitubercular drugs such as Streptomycin, Isoniazid, Rifampin, and Ethambutol (Table 2) performed according to international protocols[22,23] showed that three strains (H37Rv, SS 5, and SS10) were sensitive to all drugs, whereas SS2 (Rifampin), SS3 (Ethambutol), SS6 (Rifampin), and SS7 (Streptomycin) were resistant to a single drug (in bracket). In addition other strains SS8 and SS11 were resistant to two drugs (Streptomycin–Isoniazid and Ethambutol–Isoniazid, respectively) and eventually SS9 to three (Streptomycin, Isoniazid, and Ethambutol). Compounds **3a**,**c**,**i**,**j**,**p** and **1o** showed MIC_{90} values in the range 0.5–3.2 μg/mL, while compounds **3b**,**d**,**e–h**,**k–o** were inactive exhibiting MIC_{90} > 32 μg/mL. Derivative (**3c**) was the most potent exhibiting MIC_{90} = 0.5 μg/mL against all strains tested.

The latter derivative (**3c**) was selected as lead compound for further experiments. Human macrophages



Scheme 1. Synthesis of 3-methyl-9-substituted-6-oxo-6,9-dihydro-3*H*-[1,2,3]-triazolo[4,5-*h*]quinoline-7-carboxylic acids and ethyl esters (**3a**–**p**). Reagents and conditions: (a) HNO_3 96%, H_2SO_4 concd, 60 °C, 1 h; (b) $(CH_3)_2SO_4$, NaOH 2N, rt, 30 min; (c) H_2 45 psi, Pd/C 10%, rt, 3 h; (d) EMME, Dowtherm, 150 °C, 7 h; (e) Dowtherm, 250 °C, 10 min; (f) NaH (50%), DMF, rt, 90 °C, 0.5–20 h; NaOH 2 N, 100 °C, 2 h.

Table 1. Sensitive concentration (μg/mL) of the most active triazoloquinolones (**1o**,**3a**,**c**,**i**,**j**,**p**) and reference drug (Rifampin) against SS1-11 and H37Rv strains

M. Tuberculosis strains	**1o**	**3a**	**3c**	**3i**	**3j**	**3p**	Rifampin
SS1	1.6	0.5	0.5	0.5	1.6	0.8	>4.0
SS2	0.8	0.5	0.5	0.5	1.6	0.8	>4.0
SS3	3.2	0.5	0.5	0.5	1.6	3.2	0.5
SS4	3.2	2	0.5	2	3.2	3.2	0.7
SS5	1.6	0.5	0.5	0.5	3.2	3.2	0.5
SS6	1.6	0.5	0.5	0.5	1.6	3.2	>4.0
SS7	3.2	0.5	0.5	0.5	3.2	3.2	0.8
SS8	1.6	0.5	0.5	0.5	1.6	0.8	0.5
SS9	1.6	0.5	0.5	0.5	1.6	0.8	0.5
SS10	1.6	0.5	0.5	0.5	1.6	3.2	0.8
SS11	1.6	0.5	0.5	0.5	1.6	0.8	0.5
H37Rv	1.6	0.5	0.5	0.5	1.6	0.8	0.7

J774-A1 were infected with H37Rv strain and successively grown in the absence of antitubercular agent or in the presence of **3c** at both the concentrations of 0.5 and 0.25 μg/mL. After 7 days the macrophages were lysed and the growth of mycobacterial culture was of 5000 and 8000 CFU/mL, respectively, while the untreated culture grew regularly. Cytotoxicity of **3c** was tested against both human macrophages and Hep-2 cells and the corresponding CC_{50} values were in both cases higher than 50 μg/mL.

Preliminary structure activity relationship (SAR) studies suggest that in general the presence of an alkyl substituent at *N*-9 position (**1o** and **3a**,**c**,**i**,**j**) was more favorable than propenyl or benzyl groups, while a phenylethyl group was tolerated (**3p**).

Table 2. Breakpoint concentrations of Streptomycin, Isoniazid, Rifampin, and Ethambutol against SS1-11 and H37Rv strains

M. Tuberculosis strains	Streptomycin (1 μg/mL)	Isoniazid (0.1 μg/mL)	Rifampin (1 μg/mL)	Ethambutol (5.0 μg/mL)
SS1	**R**	S	**R**	S
SS2	S	S	**R**	S
SS3	S	S	S	**R**
SS4	**R**	**R**	S	**R**
SS5	S	S	S	S
SS6	S	S	**R**	S
SS7	**R**	S	S	S
SS8	**R**	**R**	S	S
SS9	**R**	**R**	S	**R**
SS10	S	S	S	S
SS11	S	**R**	S	**R**
H37Rv	S	S	S	S

R, resistant; S, sensitive.

In summary, our experiments demonstrated that 3-methyl-9-substituted-6-oxo-6,9-dihydro-3*H*-[1,2,3]-triazolo[4,5-*h*]quinolone-7-carboxylic derivatives are endowed with an excellent activity against MDR *M. tuberculosis* strains associated with no cytotoxicity. Further in vivo studies and SAR investigations will be reported in due course.

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