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Synthesis, antitubercular activity, and SAR study of N-substituted-phenylamino-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazides

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ABSTRACT

Tuberculosis treatment remains a challenge that requires new antitubercular agents due to the emergence of multidrug-resistant *Mycobacterium* strains. This paper describes the synthesis, the antitubercular activity and the theoretical analysis of N-substituted-phenylamino-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazides (**8a–b**, **8e–f**, **8i–j** and **8n–o)** and new analogues (**8c–d**, **8g–h**, **8l–m** and **8p–q**). These derivatives were synthesized in good yields and some of them showed a promising antitubercular profile. Interestingly the N-acylhydrazone (*NAH*) **8n** was the most potent against the *Mycobacterium tuberculosis* H37Rv strain (MIC = 2.5 μg/mL) similar to or better than the current drugs on the market. The theoretical structure–activity relationship study suggested that the presence of the furyl ring and the electronegative group (NO_2) as well as low lipophilicity and small volume group at R position are important structural features for the antitubercular profile of these molecules. NMR spectra, IR spectra and elemental analyses of these substances are reported.

1. Introduction

Tuberculosis (TB) is an infection caused by *Mycobacterium tuberculosis* with high mortality rates.[1–3] Currently, tuberculosis is endemic throughout the world, and HIV-associated TB, inadequate public health resources,[2,4] extreme poverty and the development of *M. tuberculosis* resistance to commonly used first-line drugs (i.e., isoniazid and rifampin)[6–11] are factors that contribute to the disease resurgence.[5]

TB therapy is a multi-drug regimen over a long period of time. The failure of patients to complete or manage the therapy has led to the emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB). MDR-TB is defined as the bacterial resistance to at least isoniazid and rifampicin, the most potent antibiotics used against the disease[2,6,8] whereas XDR-TB is the resistance to at least rifampicin and isoniazid in addition to any fluoroquinolone and to at least one of the three injectable drugs commonly used in antitubercular treatment (capreomycin, kanamycin and amikacin).[6,13]

Currently MDR-TB and XDR-TB are detected worldwide, including in Eastern Europe and in Africa.[12] XDR-TB is associated with a higher mortality rate than MDR-TB due to the limited options for effective treatment. This treatment deficiency is considered a serious public health threat, especially in populations already stricken by HIV and therefore more susceptible to TB infection.[6,13,14] Thus these strains, particularly XDR-TB, seriously compromises TB control efforts.[12]

Currently, there is an overwhelming need to develop new structural classes of antitubercular agents that allow shorter and more effective therapies.[1,15,16] N-acylhydrazones (*NAH*) **1–3**[17–20] and several 1,2,3-triazole analogs **3–7**[10,21–23] have been reported with antitubercular activity (Fig. 1). The studies have showed that the hydrazone group plays an important role for the antitubercular profile of compounds **1–3**.[17–20] Simultaneously, compounds containing 1,2,3-triazoles are among several heterocycles with broad medicinal applications, such as dopamine D2 receptor ligands, for treating schizophrenia,[24] antiviral,[25] antineoplastic,[26] antiplatelet,[27] trypanocidal[28] and antiophidic agents.[29]

In this work, we designed new derivatives exploring the introduction of different aromatic rings (e.g., phenyl, bromothiophenyl, furyl and nitrofuryl) attached to the imine group to verify the effect of a pharmacophoric group at the C-4 position of the 1,2,3-triazole

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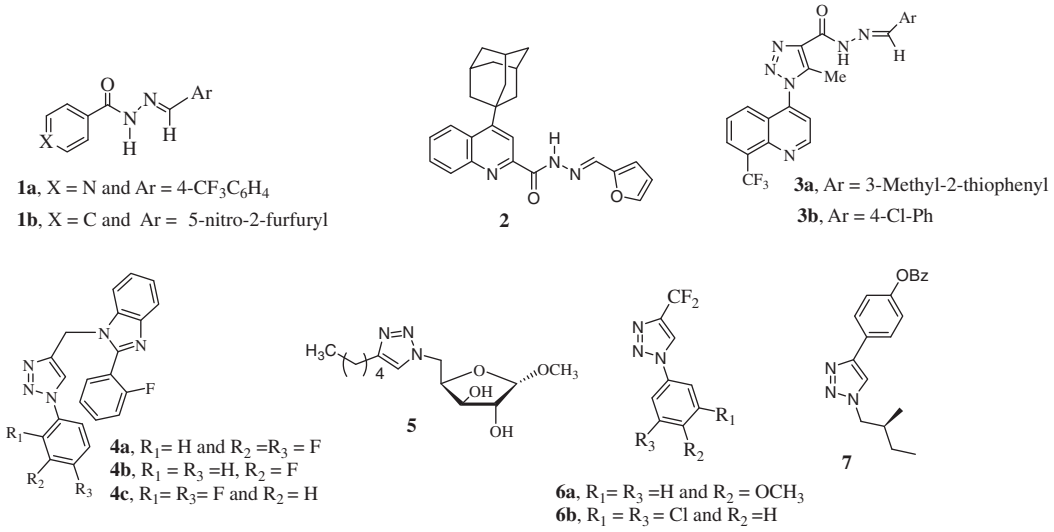


Figure 1. Tuberculostatic agents **1**–**7**.

ring. In rational design research area, halogens are known for inductive, conjugative, steric, and/or electronic effects that can be directly involved in the biological activity (i.e., antimicrobial).[30] The replacement of a halogen atom at C–O or C–H centers especially in the medicinal chemistry area can produce marked results and unexpected biological activity.[30] Therefore we synthesized the new bromo- and fluoro substituted analogues **8c–d**, **8g–h**, **8l–m** and **8p–q** to evaluate the contribution of the steric and electronic effects of these halogens for their antitubercular activity.

We also described the antitubercular profile against *M. tuberculosis* H37Rv and the structure–activity relationship (SAR) study using a molecular modeling approach of the 4-acylhydrazone derivatives[27] **8a–b**, **8e–f**, **8i–j**, **8n–o** and of the new analogs N-substituted-phenylamino-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazides **8c–d**, **8g–h**, **8l–m** and **8p–q** (Fig. 2) to identify structural features important for the biological activity.

2. Results and discussion

2.1. Chemistry

The synthesis of the 4-acylhydrazone derivatives **8a–b**, **8e–f**, **8i–j**, **8n–o,** and of the new analogues **8c–d**, **8g–h, 8l–m**, **8p–q** is shown in Scheme 1. The carbohydrazides **10a–d** were prepared in moderated yields by the condensation of the corresponding 4-carbethoxy-triazole derivatives **9a–d** with hydrazine hydrate in refluxing ethanol according to the method described in our previous report.[27]

The *NAH* derivatives **8a–b**, **8e–f** and **8i–j** as well as the novel compounds **8c–d**, **8g–h** and **8l–m** (Table 1) were prepared by the condensation of compounds **10a–d** with suitable aromatic aldehydes (Route I) in ethanol using hydrochloric acid as the catalyst (Route I). The 5-nitrofuran derivatives **8n–q** were obtained by the condensation of compounds **10a–d** with commercially available 5-nitro-2-furfurylidene diacetate in a 1:1 mixture of ethanol and sulfuric acid (Route II).

The stereochemistry of the imine double bond in this series was determined for the understanding of the biological results. A detailed analysis of the ^{1}H NMR spectra of the derivatives **8a–q** revealed only one N–*H* signal for each compound, which was attributed to the (*E*)-diastereomer, on the basis of a nuclear Overhauser (nOe) experiment. For example, irradiation of the N–*H* hydrogen signal at 10.22 ppm of compound **8n** resulted in a NOE-enhancement of the N=C*H* hydrogen signal at 8.54 ppm (14.4%) (Fig. 3). Despite the possibility of the formation of two diastereoisomers, only the (*E*)-isomer was isolated from the reaction.

2.2. In vitro and in silico analyses of the antitubercular profile against *Mycobacterium tuberculosis* H37Rv – Structure–activity relationship (SAR) study

Initially, we tested the compounds **8a–q** against *M. tuberculosis* H37Rv (ATCC 27294) at a screening concentration (100 μg/mL). These data revealed an antitubercular profile only for the acylhydrazone derivatives **8e** and **8i–q** (Table 2)**.** To analyze the importance of the carbohydrazide functional group at the C-4 position

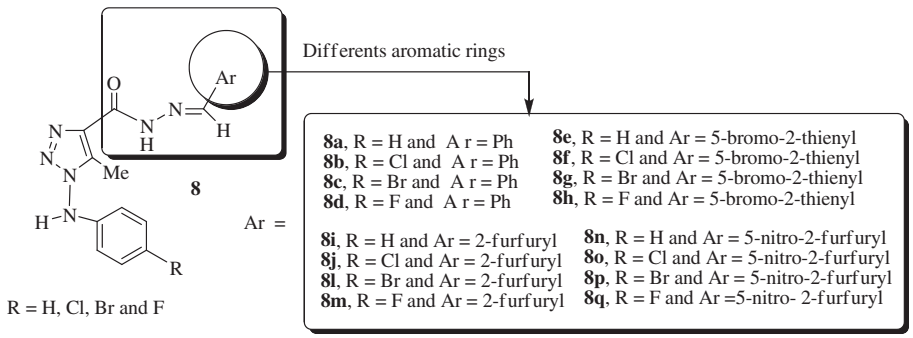
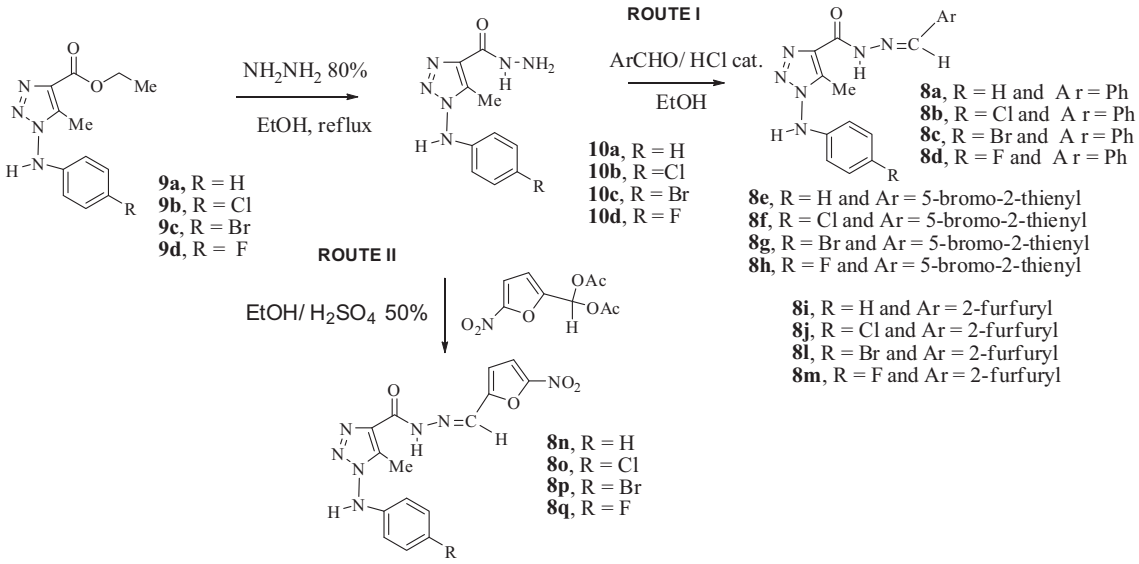
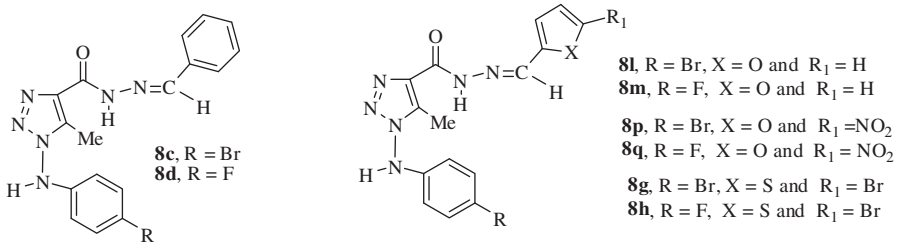


Figure 2. Strategy for the synthesis of compounds **8a–q**.



Scheme 1. Synthetic pathways used for the synthesis of compounds **8a–q**.

Table 1
Yields and melting points of *NAH* derivatives **8c–d**, **8g–h**, **8l–m** and **8p–q**



Compound	R	X	R_1	Mp (°C)	Yield (%)
8c	Br	—	—	239–240	99
8d	F	—	—	217–218	91
8g	Br	S	Br	235–238	90
8h	F	S	Br	215–218	53
8l	Br	O	H	240–242	97
8m	F	O	H	210–214	81
8p	Br	O	NO_2	177–179	80
8q	F	O	NO_2	157–165	86

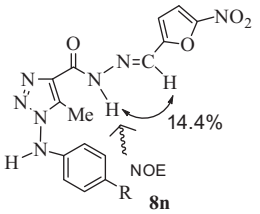


Figure 3. (*E*)-configuration of N=C bond in the derivative **8n**.

of the triazole ring, we also tested the compounds **10a–d** used to preparation of the *NAH* derivatives **8a–q**. However no effect was detected against *M. tuberculosis* H37Rv for any derivative evaluated (not shown). This result suggests that the presence of the carbohydrazide group at C-4 position of the triazole ring do not determine the biological activity *per se*.

Since the compounds **8a–d** and **8f–h** did not show any antitubercular activity (MIC >100 μg/mL), only the active compounds (**8e** and **8i–q**) were further tested for determination of the minimum inhibitory concentration (MIC) against *M. tuberculosis* H37Rv using broth macrodilution assays (Table 2). Interestingly, MIC assays of the triazole series **8a–q** revealed the nitrofuran analog **8n** as the most active compound (MIC = 2.5 μg/mL) with an antitubercular activity similar to clinical successful drugs (i.e., ethambutol MIC = 2 μg/ml) or those still in evaluation (i.e., isoxyl MIC = 2.5 μg/ml).[31] The derivatives **8i**, **8o** and **8q** also showed a potential profile (MIC = 5.0 μg/mL) as well as **8j**, **8l** and **8m** (MIC = 12.5–25 μg/mL) comparable or better than some compounds reported in the literature such as ethambutol derivative SQ109 and pyrazinamide (MIC range in 5–64 μg/mL and 12.5–25 μg/mL respectively).[2]

In this work we performed a structure–activity relationship (SAR) analysis of the compounds **8a–q** using a molecular modeling approach. Overall, the structural analysis of the 3-D structure of these derivatives revealed a conserved conformation for all acylhydrazone derivatives (Fig. 4A). This result pointed to other structural parameters, instead of only the original conformation as important to determine the antitubercular profile. Interestingly, the in vitro and in silico analyses pointed the nature of the arylhydrazone

Table 2
Comparison of the antitubercular activity of compounds **8a–q** (Minimal Inhibitory Concentration - MIC) against *Mycobacterium tuberculosis* H37Rv strain with the derivatives electronic properties (Dipole moment, HOMO and LUMO energy and number of Hydrogen Bond Acceptor - HBA and Donor - HBD groups) and other structural and stereo features (Molecular Volume, *c* log *P*, Molecular weight–Mwt, and Polar Surface Area–PSA). The most active compound of each subgroup is in bold.

#	R	X	R_1	MIC (μg/ml)	E_{LUMO} (eV)	E_{HOMO} (eV)	Dipole (debye)	Volume (Å^3)	*c* log *P*	Mwt (amu)	HBA	HBD	Σ*	PSA (Å^2)
8a	H	—	—	>100	2.41	−8.26	3.61	326.69	4.07	320.4	7	2	9	73.9
8b	Cl	—	—	>100	2.33	−8.32	2.88	340.05	4.68	354.8	7	2	9	73.7
8c	Br	—	—	>100	2.32	−8.32	2.88	345.00	4.77	399.3	7	2	9	73.7
8d	F	—	—	>100	2.36	−8.3	2.97	331.41	4.13	338.3	7	2	9	73.9
8e	H	S	Br	12.5	1.86	−8.02	5.38	331.93	4.88	405.3	8	2	10	73.8
8f	Cl	S	Br	>100	1.80	−8.08	4.27	345.28	5.49	439.7	8	2	10	73.7
8g	Br	S	Br	>100	1.79	−8.08	4.19	350.24	5.57	484.2	8	2	10	73.7
8h	F	S	Br	>100	1.82	−8.06	4.49	336.66	4.93	423.3	8	2	10	73.8
8i	H	O	H	5	2.29	−7.71	3.43	305.89	3.13	310.3	8	2	10	81.7
8j	Cl	O	H	12.5	2.21	−7.77	2.55	319.24	3.79	344.8	8	2	10	81.6
8l	Br	O	H	25.5	2.20	−7.77	2.55	324.20	3.87	389.2	8	2	10	81.6
8m	F	O	H	12.5	2.24	−7.76	2.64	310.61	3.23	328.3	8	2	10	81.7
8n	H	O	NO_2	2.5	0.98	−8.61	10.01	327.61	4.08	355.3	11	2	13	121.6
8o	Cl	O	NO_2	5	0.93	−8.67	8.88	340.96	4.14	389.8	11	2	13	121.5
8p	Br	O	NO_2	6.25	0.93	−8.68	8.78	345.90	4.78	434.2	11	2	13	121.5
8q	F	O	NO_2	5	0.95	−8.65	8.90i	332.34	3.58	373.3	11	2	13	121.6

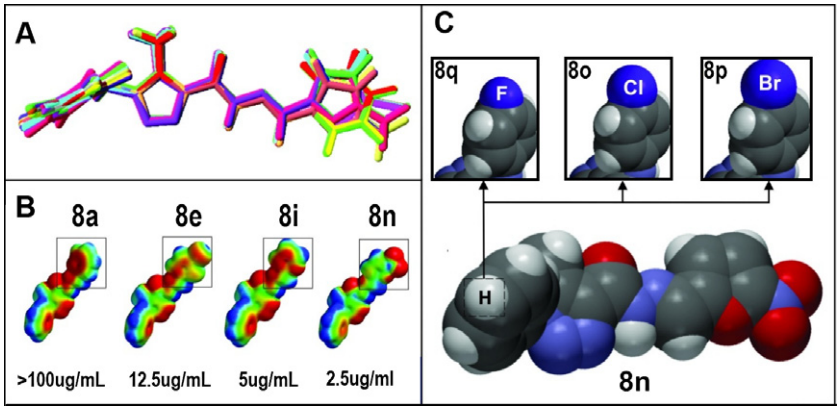


Figure 4. Structural comparison of the N-substituted-phenylamino-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazides (**8a–q**). (**A**) Superimposition of all compounds 3-D-structure revealing their similar conformation. (**B**) Comparison of the Electrostatic Potential Maps (MEP) of the most active derivative of each subgroup substituted with phenyl (**8a**), 5-bromo-2-thienyl (**8e**), furyl (**8i**) or nitrofuryl (**8n**) ring at C-2 (boxed). The analysis considering the biological activity (MIC in μg/mL) showed the less diffused and more oriented charge distribution related to the best antitubercular profile. All compounds are at same orientation, negative and positive charges are represented by red and blue respectively and range (-70 – 90 kJ/mol). (**C**) Comparison of the halogen substitution of **8n** subgroup showing the volume of each halogen that negatively affects the biological activity (insets).

moiety as important to improve the biological activity (Table 2 and Fig. 4). In fact, both replacements of the bromothiophenyl ring (e.g., **8e–h**) by a furyl or a nitrofuryl moiety (e.g., **8i–m** and **8n–q**, respectively) enhanced the antitubercular activity. According to the electrostatic potential map analysis, these substitutions led to a singular electrostatic potential distribution that specifically oriented the positive and negative charges and probably allowed more interactions with the bacterial target (Fig. 4B).

In Drug design, halogen atoms are used to improve penetration through lipid membranes and tissues. They may also present a significant reactivity depending on the structure of the molecule. In this work, the introduction of a *para*-halogen substituent in the *N*-phenyl ring of **8e** (e.g., **8f–h**), **8i** (e.g., **8j–m**) or **8n** (e.g., **8o–q**) presented a negative effect as they reduced the antitubercular activity (Table 2). Probably, the halogen reactivity at R position compromised the original interactions of the non-substituted compounds with the bacterial target. The in silico analysis also pointed the volume of the halogen as a feasible restrictive factor since bromine, the largest halogen, is more deleterious to the antitubercular activity than chlorine or fluorine (Fig. 4). Based on these data we may infer that R is a steric and/or restricted position that should be carefully considered in the future design of antitubercular agents (Fig. 5).

Herein we also calculated the stereoelectronic properties of these derivatives including HOMO and LUMO energy, coefficient distribution and density, lipophilicity (*c* log *P*), molecular volume, molecular area, polar surface area, number of hydrogen bounds donors (HBD) and acceptors (HBA) groups, solubility and dipole moment (Table 2). Interestingly, we noticed an analogous behavior for the most active compounds of each subgroup. All of them presented the highest values of HOMO and LUMO energy and dipole moment, and the lowest volume, molecular weight and *c* log *P* of its own subgroup (Table 2, in bold). Thus, low lipophilicity, less steric and smaller reactive groups should to be preferential for designing new antitubercular molecules. More importantly, these data suggested that more than one parameter, simultaneously and all together, modulate the activity profile of these compounds. Therefore the difficult of finding new antitubercular agents may be due to the complexity of planning an active molecule that fulfills all these structural requirements.

The analysis of the biological data of each subgroup showed that the derivatives with the nitro group at furyl ring C-5 position (**8n–q**) presented the best antibacterial profile (MIC = 2.5–12.5 μg/mL) (Table 2). According to our SAR, the active biological profile of the **8n** subgroup is due to different factors including the increase of the number of hydrogen acceptors and dipole moment allowed

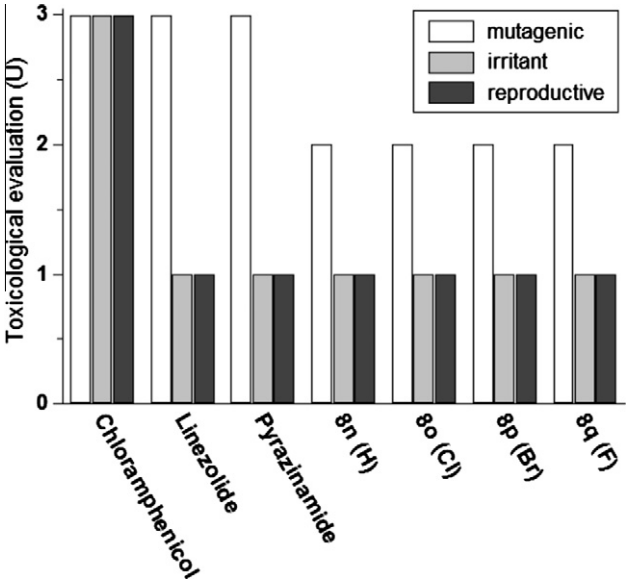


Figure 5. Comparison of the theoretical toxicity risks of the most active subgroup (**8n–q**) with commercial drugs. The risks of mutagenicity, irritability and reproductive negative effects are represented as numbers 1 (low), 2 (medium) or 3 (high).

by the addition of the nitro group. These chemical features probably increased the receptor-ligand interactions and/or the compounds binding affinity. In addition, the reduced LUMO energy values of this subgroup points to a better electrophilic profile that may help in orienting and/or interacting with the bacterial target.

The SAR studies involving the electrostatic potential maps (MEP) of the compound **8n** showed that the presence of a nitro group generated a singular molecular electrostatic distribution. The MEP revealed a strong electronic charge in the C-2 position of the furan ring and in the nitro group at C-5 position (Fig. 4B) for all compounds of this subgroup, which seems to be relevant for the antitubercular activity.

The theoretical toxicity risks study (mutagenic, tumorigenic, and irritant) of the N-substituted-phenylamino-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide derivatives revealed a lower theoretical toxicity profile for all compounds of the most active series (**8n–q**) in comparison with the commercial drug Chloramfenicol and similar to others such as Linezolide (Fig. 5). Literature[32] describes nitrofuran group as possibly involved in derivatives toxicity profile. Since other compounds without the 5-nitrofuran group attached to the acylhydrazone framework of the triazole ring were also active in this work, this group is not essential for the antitubercular activity and may be replaced to optimize these molecules. Thus, we propose the exchange of the nitrofuran by a furan ring substituted in the 5-position with a sulfonamide functional moiety, which is described in the literature[33] as an antimicrobial group, in attempt to modulate the toxicity profile. In addition, since our theoretical study also pointed the phenylamino group at N-1 position of the 1,2,3-triazole ring as probably involved in the detection of the mutagenic theoretical profile (not shown), we also suggest its substitution by benzylamino or benzyl(phenyl)amino group. According to our theoretical evaluation, these groups decreased the derivative mutagenic effect, leading to a safer toxicity theoretical profile.

Interestingly, our theoretical studies showed that all compounds fulfilled Lipinski's 'Rule of five' (*c* log $\leq$5, molecular weight $\leq$500, number of hydrogen bond donors $\leq$5 and number of hydrogen-bond acceptors $\leq$10)[34] (Table 2), also meeting at least one criterion of those proposed by Veber, (number of rotation bonds $\leq$10 and polar surface area $\leq$140 Å^2)[35] (Table 2). This result reinforced the promising profile of these compounds for further experimental in vivo and in vitro biodisponibility investigation.

3. Conclusions

In summary, we reported the synthesis and the in vitro and in silico evaluations of the N-substituted-phenylamino-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazides **8a–b**, **8e–f**, **8i–j**, **8n–o** as well as of the new analogs **8c–d**, **8g–h** and **8p–q** as antitubercular agents. The *NAH* compounds **8a–d** (Ar = phenyl) did not show any antitubercular profile whereas the acylhydrazones **8n–q** (Ar = nitrofuryl) were more active than their corresponding analogs in the series **8a–d** (Ar = phenyl), **8e–h** (Ar = bromothiophenyl) and **8j–m** (Ar = furyl). Among the nitrofuran derivatives, the non-substituted compound **8n** was the most potent molecule and exhibited a MIC value (2.5 μg/mL) comparable to other clinically successful drugs such as ethambutol (MIC = 2 μg/mL). The SAR study pointed the presence of the furyl ring, the electronegative group (NO_2), the low lipophilicity and small volume groups at R position as important structural features for the antitubercular profile of these compounds. These parameters should be further considered in future design of new and more effective compounds and drug evaluations.

4. Experimental

Melting points were determined with a Fisher–Johns instrument and are reported uncorrected. Infrared (IR) spectra were recorded on an ABB FTLA2000–100 spectrophotometer in KBr pellets. NMR spectra, unless otherwise stated, were obtained in Me_2SO-d_6 or $CDCl_3$ using a Varian Unity Plus 300 MHz spectrometer. Chemical shifts (δ) are reported in ppm and the coupling constant (*J*) in Hertz. Column chromatography purification was performed on flash silica gel from Acros Organics. Reactions were routinely monitored by thin layer chromatography (TLC) on silica gel pre-coated F_{254} using Merck plates. Microanalyses were performed on a Perkin–Elmer Model 2400 instrument, and all reported values were within ±0.4% of the calculated compositions.

4.1. Chemistry

4.1.1. General procedure for the preparation of the N-substituted-phenyltriazolyl-4-acylhydrazone derivatives 8c, 8d, 8g, 8h, 8l, 8m, 8p and 8q

To a solution of hydrazide derivatives **10c–d** (0.50 mmol) in 15 mL of EtOH, an equimolar amount of the appropriate aromatic aldehyde was added in the presence of a catalytic amount of HCl (37% v/v). The reaction was stirred for 2 h at room temperature. The solvent was then evaporated, and the precipitate was collected by filtration, washed with cold water and dried under vacuum. The *NAH* derivates **8c, 8d, 8g, 8h, 8l, 8m, 8p and 8q** were purified by a flash chromatography column using *n*-hexane/EtOAc (1:1) as the eluent.

4.1.2. 1-(4''-Bromophenylamino)-5-methyl-*N*′-(phenylmethylene)-1*H*-[1,2,3]-triazole-4-carbohydrazide 8c

Compound **8c** obtained as a white solid by condensation of **10c** with benzaldehyde. IR (KBr) ν_{max} (cm^{-1}) 3439 and 3197 (N–H); 1646 (C=O); 1603 (C=N) ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.47 (s, 3H, C*H*$_3$), 6.47 (d, 2H, *J* = 8.8, H-2' and H-6'), 7.42–7.47 (m, 5H, H-2'', H-3'', H-4'', H-5'' and H-6''), 7.71 (d, 2H, *J* = 8.8, H-3' and H-5'), 8.58 (s, 1H, N=C*H*), 10.36 (br s, 1H, N–*H*), 12.10 (br s, 1H, N*H*–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.1 (*C*H$_3$), 112.8 (C-4'), 115.1 (C-2' and C-6'), 127.1 (C-3'' and C-5''), 128.8 (C-2'' and C-

6''), 130.1 (C-4''), 132.1 (C-3' and C-5'), 134.3 (C-1'') 136.3 (C-4 or C-5), 138.4 (C-4 or C-5), 145.4 (C-1'), 148.2 (N=*C*H), 156.9 (C=O) ppm. Anal. Calcd for $C_{17}H_{15}BrN_6O$: C, 51.14; H, 3.79; N, 21.05. Found: C, 51.10; H, 4.00; N, 20.41.

4.1.3. 1-(4''-Fluorophenylamino)-5-methyl-*N*′-(phenylmethylene)-1*H*-[1,2,3]-triazole-4-carbohydrazide 8d

Compound **8d** obtained as a white solid by condensation of **10d** with benzaldehyde. IR (KBr) ν_{max} (cm^{-1}) 3304 and 3054 (N–H); 1676 (C=O); 1592 (C=N) ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.48 (s, 3H, C*H*$_3$), 6.54 (dd, 2H, *J* = 8.8; 4.4, H-2' and H-6'), 7.11 (dd, 2H, *J* = 8.8; 4.4, H-3' and H-5'), 7.45–7.47 (m, 3H, H-2'', H-4'' and H-6''), 7.68–7.75 (m, 2H, H-3'' and H-5''), 8.58 (s, 1H, N=C*H*), 10.36 (br s, 1H, N–*H*), 12.10 (br s, 1H, N*H*–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.1 (*C*H$_3$), 114.6 (*J* = 8.5, C-2' e C-6'), 116.0 (*J* = 22.6, C-3' and C-5'), 127.0 (C-3'' and C-5''), 128.8 (C-2'' and C-6''), 130.0 (C-4''), 134.3 (C-1''), 136.2 (C-4 or C-5), 138.3 (C-4 or C-5), 142.6 (*J* = 2.3, C-1'), 148.1 (N=*C*H), 157.0 (C=O) ppm. Anal. Calcd for $C_{17}H_{15}FN_6O$: C, 60.35; H, 4.47; N, 24.84. Found: C, 58.13; H, 4.69; N, 24.13.

4.1.4. *N*′-[(5′′-Bromothien-2′′-yl)methylene]-1-(4'-bromophenylamino)-5-methyl-1*H*-[1,2,3]-triazole-4-carbohydrazide 8g

Compound **8g** obtained as a white solid by condensation of **10c** with 2-bromothiophene-5-carboxaldehyde. IR (KBr) ν_{max} (cm^{-1}) 3298 and 3165 (N–H); 1640 (C=O); 1602 (C=N) ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.45 (s, 3H, C*H*$_3$), 6.46 (d, 2H, *J* = 8.8, H-2' and H-6'), 7.27 (d, 1H, *J* = 0.7, H-2''), 7.27 (d, 1H, *J* = 0.7, H-3''), 7.42 (d, 2H, *J* = 8.8, H-3' and H-5'), 8.66 (s, 1H, N=C*H*), 10.38 (br s, 1H, N–*H*), 12.23 (br s, 1H, N*H*–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.4 (*C*H$_3$), 113.3 (C-4'), 115.3 (C-4''), 115.4 (C-2' and C-6'), 131.7 (C-2''), 131.9 (C-3''), 132.6 (C-3' and C-5'), 136.4 (C-4 or C-5), 139.1 (C-4 or C-5), 141.1 (C-1''), 142.9 (N=*C*H), 145.6 (C-1'), 157.4 (C=O) ppm. Anal. Calcd for $C_{15}H_{12}Br_2N_6OS$: C, 37.21; H, 2.50; N, 17.36. Found: C, 37.68; H, 3.01; N, 16.81.

4.1.5. *N*′-[(5′′-Bromothien-2′′-yl)methylene]-1-(4'-fluorophenylamino)-5-methyl-1*H*-[1,2,3]-triazole-4-carbohydrazide 8h

Compound **8h** obtained as a white solid by condensation of **10d** with 2-bromothiophene-5-carboxaldehyde. IR (KBr) ν_{max} (cm^{-1}) 3439 and 3292 (N–H); 1664 (C=O); 1601 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.46 (s, 3H, C*H*$_3$), 6.56 (d, 2H, *J* = 8.8; 4.5, H-2' and H-6'), 7.10 (dd, 2H, *J* = 8.8; 4.5 H-3' and H-5'), 7.27 (m, 2H, H-2'' and H-3''), 8.66 (s, 1H, N=C*H*), 10.17 (br s, 1H, N–*H*), 12.17 (br s, 1H, N*H*–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.0 (CH$_3$), 114.6 (C-4''), 114.7 (*J* = 7.7, C-2' and C-6'), 115.9 (*J* = 23.2, C-3' and C-5'), 131.1 (C-2′′), 131.2 (C-3′′), 136.1 (C-4 or C-5), 138.3 (C-4 or C-5), 141.0 (C-1′′), 142.1 (N=CH), 142.4 (C-4'), 156.8 (C=O) ppm. Anal. Calcd for $C_{15}H_{12}BrFN_6OS$: C, 42.56; H, 2.86; N, 19.86. Found: C, 42.13; H, 2.79; N, 18.63.

4.1.6. 1-(4'-Bromophenylamino)-*N*′-[(fur-2′′-yl)methylene]-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide 8l

Compound **8l** obtained as a white solid by condensation of **10c** with 2-furaldehyde. IR (KBr) ν_{max} (cm^{-1}) 3437 and 3220 (N–H); 1671 (C=O); 1589 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.46 (s, 3H, C*H*$_3$), 6.47 (d, 2H, *J* = 8.8, H-2' and H-6'), 6.63 (dd, 1H, *J* = 2.9; 1.7, H-3''), 6.90 (d, 1H, *J* = 2.9, H-2''), 7.42 (d, 2H, *J* = 8.8, H-3' and H-5'), 7.85 (d, 1H, *J* = 1.7, H-4''), 8.46 (s, 1H, N=C*H*), 10.39 (br s, 1H, N–*H*), 12.15 (br s, 1H, N*H*–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.3 (*C*H$_3$), 112.4 (C-3''), 113.1 (C-4'), 113.8 (C-2''), 115.2 (C-2' and C-6'), 132.3 (C-3' and C-5'), 136.4 (C-4 or C-5), 138.2 (N=*C*H), 138.2 (C-4''), 138.7 (C-4 or C-5), 145.6 (C-1'), 149.6 (C-1''), 157.6 (C=O) ppm. Anal. Calcd for $C_{15}H_{13}BrN_6O_2$: C, 46.29; H, 3.37; N, 21.59. Found: C, 46.59; H, 3.91; N, 20.69.

4.1.7. 1-(4'-Fluorophenylamino)-*N*′-[(fur-2′′-yl)methylene]-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide 8m

Compound **8m** obtained as a white solid by condensation of **10d** with 2-furaldehyde. IR (KBr) ν_{max} (cm^{-1}) 3297 and 3195 (N–H); 1645 (C=O); 1607 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.47 (s, 3H, C*H*$_3$), 6.54 (d, 2H, *J* = 8.8; 4.4, H-2' and H-6'), 6.64 (dd, 1H, *J* = 3.4; 1.7, H-3''), 6.90 (d, 1H, *J* = 3.4, H-2''), 7.10 (d, 2H, *J* = 9,0; 8.8, H-3' and H-5'), 7.85 (d, 1H, *J* = 1.7, H-4''), 8.46 (s, 1H, N=C*H*), 10.20 (br s, 1H, N–*H*), 12.14 (br s, 1H, N*H*–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.1 (*C*H$_3$), 112.1 (C-3''), 113.2 (C-2''), 114.6 (*J* = 7.7, C-2' and C-6'), 115.9 (*J* = 23.2, C-3'and C-5'), 136.2 (C-4 or C-5), 137.8 (N=*C*H), 137.8 (C-4''), 138.3 (C-4 or C-5), 142.5 (*J* = 1.7, C-1'), 149.5 (C-1''), 156.8 (C=O), 157.2 (*J* = 236.9, C-4') ppm. Anal. Calcd for $C_{15}H_{13}FN_6O_2$: C, 54.88; H, 3.99; N, 25.60. Found: C, 54.91; H, 3.99; N, 25.48.

4.1.8. General procedure for the preparation of the N-substituted-phenyltriazolyl-4-acylhydrazone derivatives 8p–q

A solution of 5-nitro-2-furfurylidene diacetate (0.4 mmol) in a mixture of EtOH (10.0 mL) with a 50% aqueous solution of sulfuric acid (1.0 mL) was heated for 1–2 min on a steam bath and cooled to room temperature. Compounds **10c–d** (0.4 mmol) were then added and the resulting mixture was stirred for two hours at room temperature after which it was poured into crushed ice. The insoluble product was filtered off and then purified by column chromatography using a 1:1 *n*-hexane/EtOAc mixture as the eluent.

4.1.9. *N*'-[(5′′-Nitrofur-2′′-yl)methylene]-1-(4'-bromophenylamino)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide 8p

Compound **8p** obtained as a yellow by condensation of **10c** with 5-nitrofuraldehyde. IR (KBr) ν_{max} (cm^{-1}) 3435 and 3267 (N–H); 1637 (C=O); 1613 (C=N) ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.47 (s, 3H, C*H*$_3$), 6.48 (d, 2H, *J* = 8.8, H-2' and H-6'), 7.25 (d, 1H, *J* = 4.1, H-3''), 7.43 (d, 2H, *J* = 8.8, H-3' and H-5'), 7.78 (d, 1H, *J* = 3.9, H-2''), 8.53 (s, 1H, N=C*H*), 10.38 (br s, 1H, N–*H*) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.1 (*C*H$_3$), 112.8 (C-4'), 114.6 (C-2''), 114.9 (C-3''), 115.1 (C-2' and C-6'), 132.1 (C-3' and C-5'), 135.8 (N=*C*H), 135.9 (C-4 or C-5), 139.0 (C-4 or C-5), 145.2 (C-1'), 145.3 (C-1''), 151.8 (C-4''), 157.1 (C=O) ppm. Anal. Calcd for $C_{15}H_{12}BrN_7O_4$: C, 41.49; H, 2.79; N, 22.58. Found: C, 41.10; H, 2.50; N, 18.20.

4.1.10. *N*'-[(5′′-Nitrofur-2′′-yl)methylene]-1-(4'-fluorophenylamino)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide 8q

Compound **8q** obtained as a yellow by condensation of **10d** with 5-nitrofuraldehyde. IR (KBr) ν_{max} (cm^{-1}) 3200 (N–H); 1664 (C=O); 1589 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.48 (s, 3H, C*H*$_3$), 6.58 (d, 2H, *J* = 9.0, H-2' and H-6'), 7.10 (d, 2H, *J* = 9.0, H-3' and H-5'), 7.25 (d, 1H, *J* = 3.8, H-2''), 7.78 (d, 2H, *J* = 3.8, H-3''), 8.53 (s, 1H, N=C*H*), 10.21, (br s, 1H, N–*H*), 12.59 (br s, 1H, N*H*–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.2 (*C*H$_3$), 114.6 (C-2''), 114.7 (*J* = 7.7, C-2' and C-6'), 115.0 (C-3''), 116.0 (*J* = 22,6, C-3' and C-5'), 135.7 (N=*C*H), 135.8 (C-4 or C-5), 138.9 (C-4 or C-5), 142.5 (*J* = 1.7, C-1'), 151.8 (C-1''), 151.8 (C-4''), 157.2 (C=O), 157.3 (*J* = 237.5, C-4') ppm. Anal. Calcd for $C_{15}H_{12}FN_7O_4$: C, 48.26; H, 3.24; N, 26.27. Found: C, 48.10; H, 3.10; N, 26.20.

4.2. Pharmacology

4.2.1. In vitro evaluation of antitubercular activity

The drug potency was determined by the Microplate Alamar Blue assay (MABA), which shows good correlation and proportionality with the BACTEC MGIT 960 method. This colorimetric method uses the Alamar Blue–resazurin-based oxidation–reduction indicator to obtain drug susceptibility measurements for bacteria.

The minimum inhibitory concentration (MIC, μg/mL) was defined as the lowest drug concentration that prevented a color change from blue (no growth) to pink (growth). Rifampicin, ethambutol and isoniazid were used as reference drugs.

4.3. Molecular modeling and SAR studies

All molecular computations were performed using SPARTAN008 (Wavefunction Inc. Irvine, CA, 2000) as described elsewhere.[24,27] Briefly the structures were optimized to a local minimum and the equilibrium geometry obtained in vacuum using RM1 semi-empirical methods. Subsequently, molecules were submitted to a single-point energy ab initio calculation, at the 6-31G* level, to calculate some stereoelectronic properties and perform the SAR studies. Thus, we calculated HOMO and LUMO energies and density isosurface, molecular weight, molecular surface area, polar surface area, dipole moment, lipophilicity and electrostatic potential maps for all compounds best conformation. The theoretical study of bioavailability and toxicity properties was performed using Osiris Property Explorer (http://www.organic-chemistry.org/) and Molinspiration program (http://www.molinspiration.com/cgi-bin/properties) to access the theoretical toxicity risks along with drug likeness and drug score values; the Lipinski "Rule of five" which pointed $c\log P \leq 5$, molecular weight ≤ 500, number of hydrogen bond donors ≤ 5 and number of hydrogen-bond acceptors ≤ 10; and Veber criteria, pointing PSA values being lower than 140 $Å^2$ and having less rotatable bounds than 10 as important features for bioavailability.

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