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Design, synthesis and 2D QSAR study of novel pyridine and quinolone hydrazone derivatives as potential antimicrobial and antitubercular agents

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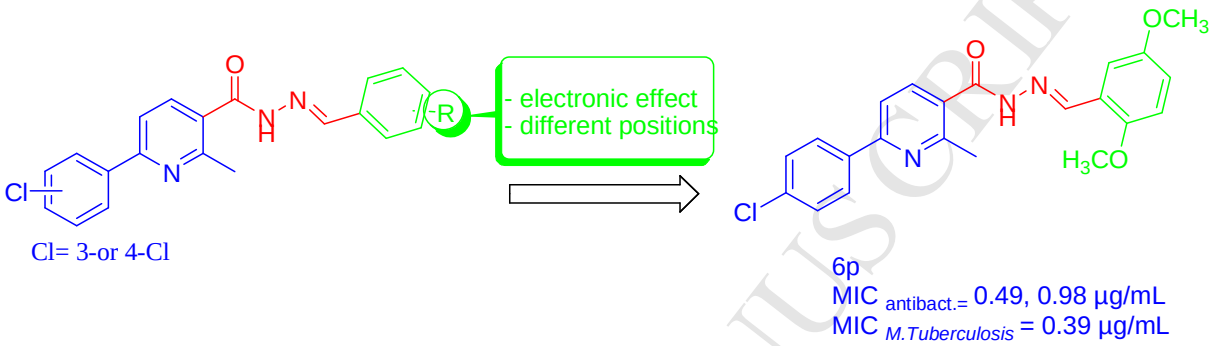
Graphical abstract

Compounds **6o**, **6p**, and **6r** displayed superior antimycobacterial (MIC = 0.39 µg/mL) and antimicrobial (MICs = 0.49- 3.9 µg/mL) activities. Compounds **6e**, **6f**, **6l** and **6n** showed very good antimycobacterial activities with MIC 0.78 µg/mL and good antimicrobial activity MICs = 0.49- 15.63 µg/mL.



1. Introduction

Tuberculosis (TB) could not be merely considered a disease, but rather a prevalent epidemic, which according to the World Health Organization (WHO), is considered as the world's deadliest communicable disease [1]. As stated in the latest WHO report, there were an estimated 10.4 million new (incident) TB cases in 2015 worldwide, of which HIV patients accounted for 1.2 million (11%) of all new TB cases, [2]. There was also an estimation of 1.4 million TB deaths in the same year, with an additional of 0.4 million deaths among HIV infected people. Although the number of TB deaths fell by 22% between 2000 and 2015, TB remained one of the top 10 causes of death worldwide in 2015 [2-4]. The development of drug resistant strains including multidrug-resistant (MDR), extremely drug resistance (XDR) and the recently cases of totally drug resistance (TDR), profoundly increase the challenges to eradicate TB worldwide [4,5]. In 2015, there were an estimated 480 000 new cases of multidrug-resistant TB (MDR-TB) [2]. Moreover, WHO estimates that one third of the world population are infected by latent TB, where treatment is still unavailable due to the lack of new drugs.

In the same context, the emergence of resistance to the major classes of antibacterial agents constitute a serious public health threat. Particularly, in recent years, much attention has been directed towards the multi-drug resistant bacteria and fungi, resulting mainly from the widespread use and misuse of classical antimicrobial drugs [6]. On the report of WHO, multidrug-resistant bacteria are responsible for approximately 25000 deaths in Europe each year [7,8]. In fact, bacterial infection has evolved once again as a global serious threat.

Consequently, one of the fundamental challenges in medicinal chemistry has become the development of new antimycobacterial and antibacterial agents with potent activity against these drug-resistant microorganisms [9]. Regarding development of novel antitubercular and antibacterial agents, quinolone derivatives and isonicotinyl hyrazone analoges arose much interest due to their well established activities and clinical applications [10-19].

Quinolones are one of the most useful and versatile antibacterial agents, where several candidates are already in clinical use such as ciprofloxacin **I**, norfloxacin **II**, it is also a core pharmacophore in the recently developed antitubercular drugs such as Bedaquiline **III**, mefloquine **IV**, moxifloxacin **V**, (Figure 1) [20-23]. In addition to novel synthesized compounds that with promising antitubercular and antibacterial agents such as **VI** [24], (Figure 1).

Moreover, several INH derivatives have also shown interesting anti-TB activities such as, saluzide **VII**, salizide **VIII**, verazide **IX**, ftivazide **X**, INHA-17 **XI**, (Figure 1) [25-33].

It is also interesting to note that, substituted carbohyrazone moiety has been reported as a well-known pharmacophoric group for antitubercular and antibacterial activity. [34-41]

Therefore, the target compounds 1,4-dihydroquinoline-3-carbohydrazide **12a-l** and substituted benzylidene-2-methylnicotinohydrazide **6a-r**, were rationalized so as to comprise these hydrazone pharmacophores. With the aim of extending the antibacterial spectrum, structural modifications on the benzenoid and the pyridine rings of quinolone, as well as the nicotinohydrazone scaffold were carried out. Furthermore, 2D-QSAR models for the novel compounds were generated in order to correlate these activities to their physically relevant descriptors.

2. Results and Discussion

2.1. Chemistry

The synthetic pathways adopted to prepare the new targeted compounds are depicted in schemes **1-2**. In scheme 1, ethyl 2-methyl-6-arylnicotinate **3a,b** was obtained *via* the reaction of enaminones **2a,b** with ethyl acetoacetate and ammonium acetate in refluxing acetic acid [42]. The nicotinic acid hydrazides **4a,b** were obtained in 70-90% yield, through hydrazinolysis of the ester derivatives **3a,b** (Scheme 1).

IR spectra of the hydrazides **4a,b** showed absorption bands due to the carbonyl group in the region 1643, 1660 cm^{-1}, in addition to bands in the region from 3282, 3321 and 3322, 3195 cm^{-1}, assigned to the NH and NH_2 groups. The ^1H NMR spectra of **4a,b** showed two singlet D_2O-exchangeable signals attributed to NH and NH_2 protons in the region δ 9.63 and 4.54 *ppm*, respectively, while the methyl ($-CH_3$) protons appeared as singlets around δ 2.50, 2.61 *ppm*. Moreover, ^{13}C NMR spectra of **4a,b** showed signals resonating around δ 166 *ppm* attributable to the carbons of carbonyl groups, while the carbons of the methyl groups appeared in δ 23.04, 23.56 *ppm*. Consequently, these hydrazide derivatives **4a** and **4b** were reacted with different aldehydes **5a-j**, in ethanol in the presence of a catalytic amount of glacial acetic acid to furnish the target derivatives **6a-r** (Scheme 1).

IR spectra of the synthetic compounds **6a-r** showed absorption bands due to the NH groups in the region 3163-3228 cm^{-1}, in addition to a carbonyl band in the region 1643-1665 cm^{-1}. The ^1H NMR spectra of **6a-r** showed two singlet D_2O-exchangeable signals attributable to NH protons of the hydrazine function (=N-NH-) in the region δ 10.70- 12.36 *ppm*, respectively, and the methyl ($-CH_3$) protons appeared as singlet signals around δ 2.51- 2.67 *ppm*, while the (C<u>H</u>=N) protons appeared as a singlet around δ 7.97-8.67 *ppm*. Furthermore, ^{13}C NMR spectra of **6a-r** showed

signals resonating in the range δ 164.15- 164.40 *ppm* attributable for the carbons of carbonyl groups, while the carbons of the methyl groups appeared in the range δ 21.43-23.44 *ppm*.

It has been reported that compounds containing arylidene ‑ hydrazide structure may exist as *E/Z* geometrical isomers about –C=N– double bond and cis/trans amide conformers around amide function (-CONH-). These compounds were present in higher percentage in DMSO-d6 in the form of geometrical E isomer about –C=N– bond [43,44]. The presence of cis/trans conformers can be established by observing the signal of the amide group (–CONH–) which appeared as two singlets. Consequently, in all cases throughout the work, two signals of azamethine of *E* isomer proton (–N=CH–) appeared at $\delta \approx$ 7.97-8.10 and 8.21-8.43 ppm [45,46] due to the formation of cis/trans conformers. In the present study, it could be suggested that the signals of *Z* isomer (which are the up-field signals [47,48] have not appeared, whereas the cis/trans conformers of *E* isomer were clearly observed.

Scheme 1

On the other hand, ethyl anilinomethylenemalonate **8a,b** was obtained via reaction of substituted aniline **7a,b** with diethyl ethoxy methylene malonate. Cyclization of the **8a,b** in diphenyl ether gave the substituted 1, 4-dihydro-4 oxoquinoline-3-carboxylic acid ethyl ester, **9a,b**. The 1,4-dihydro-4-oxo-quinoline-3-carbohydrazide derivatives **10a,b** were obtained through the hydrazinolysis of the ester derivatives **9a,b** with refluxing hydrazine hydrate with 70-80% yield. The target compounds **12a-l** were obtained through reaction of the hydrazides **10a,b** with different aldehydes in DMF (Scheme 2).

IR spectra of compounds **12a-l** showed absorption bands due to the carbonyl group in the region 1647-1680 cm^{-1}, in addition to bands in the region from 3201 to 3425 cm^{-1}, which were assigned to the NH groups. The ^{1}H NMR spectra of **12a-l** showed two singlets D_2O-exchangeable singlets attributed to –CONH and NH protons in the region δ 12.65-13.16 and 13.03 -13.28 *ppm*, respectively, while the (CH=N) protons appeared as a singlet around δ 8.31-8.67 *ppm*. ^{13}C NMR spectra of **12a-l** showed signals resonating around δ 161 *ppm* attributable to the carbons of carbonyl groups.

Scheme 2

2.2. Anti-Microbial Activity

Antibacterial and antifungal activities were performed at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Target compounds **6a–r**, **12a-l** and reference drugs were evaluated *in vitro* for their antibacterial, antifungal and anti-mycobacterial activities, by inhibition zone technique and minimum inhibitory concentration (MIC), using two fungi: *Aspergillus fumigatus* (RCMB 02568, Af) and *Candida albicans* (RCMB 05036, Ca), two gram-positive bacteria: *Streptococus pneumoniae* (RCMB 010010, Sp), and *Staphylococcus aureus* (RCMB 010028, Sa), two gram-negative bacteria: *Pseudomonas aeruginosa* (RCMB 010043, Pa), *Escherichia coli* (RCMB 010052, Ec), and *Mycobaterium tuberculosis* (RCMB 010126).

2.2.1. Anti-Fungal Activity

The results of the tested compounds against *Aspergillus fumigatus* and *Candida albicans* were presented in tables **1** and **2**, compounds **6f**, and **6p,** 2-methylnicotinohydrazide exhibited superior antifungal activities over all the synthesized target compounds. This compound **6f** demonstrated two-folds the activity against *Aspergillus fumigatus* and *Candida albicans* with concentrations 0.98 and 0.49 µg/mL, respectively, while compound **6p** showed two-folds of activity against *Aspergillus fumigatus* and the same against *Candida albicans* with concentrations 0.98 and 0.98 µg/mL, respectively, compared to concentration of amphotericin B (0.98 and 1.95 µg/mL, respectively).

Table 1.

Table 2.

These results of antifungal activities were greatly appreciated as it is well demonstrated that *Aspergillus fumigatus* cause invasive aspergillosis on immune comprised patients [49]. In addition to the well-known resistance of *Candida albicans* to antifungal agents [50]. Similarly, the trimethoxy phenyl counterpart **6l** and 2,5-dimethoxy phenyl **6r** showed significant antifungal activities against *Aspergillus fumigatus* with a concentration half the standard amphotericin B (0.98 and 1.95 µg/mL, respectively), good activity was also demonstrated against *Candida albicans*, with MIC= 1.95 µg/mL.

Compound **6a**, bearing 4-Chloro benzylidene and 2-hydroxy3-methoxy benzyl **6o** was equipotent to amphotericin B against *Aspergillus fumigatus* (MIC = 1.95 µg/mL) and showing moderate activity against *Candida albicans.* It is worth mentioning also that 4-Cl-substitution showed better

activity than the 2,4-dichloro benzylidine derivative **6b**. Moderate activities was also displayed by compound **12b** against both antifungal strains, and **12e** against *Aspergillus fumigatus.* The remarkable antifungal activity of **6f**, points out the significance of addition of trimethoxy phenyl carbohydrazide moiety and demonstrated as well better activity conferred by changing the chloro group from 4-to 3-postion.

2.2.2. Antibacterial Activity

As indicated in Tables 1 and 2, compounds **6a**, **6f**, **6l**, **6p** and **12b** displayed significant antibacterial activity against the tested gram-positive and gram-negative bacteria as compared with ampicillin and ciprofloxacin, respectively.

As regards to the antibacterial activity, compounds **6f** and **6p** comprising 3,4,5-trimethoxybenzylidene or 2,5-dimethoxy moieties elicited significant activities against both tested gram-positive and gram-negative organisms, and even showing 2-fold increased inhibition against *Streptococus pneumonia* with (MIC = 0.49 µg/mL), compared to ampicillin (MIC= 0.98 µg/mL). Besides compounds **6o, 6r** and **12b** exhibited equipotent activity to ampicillin against *Streptococus pneumonia* (MIC = 0.98 µg/mL), while compounds to **6l** and **6p** demonstrated the same activity as ampicillin against *Staphylococcus aureus* with MIC= 0.98 µg/mL. Amongst all the novel methylnicotinohydrazone series, it was observed that, compounds bearing electron-donating groups showed better activities than the electron withdrawing ones.

Comparing compound **12b** with its counterpart, **12a**, it could be assumed that the 2,4-dichlorobenzylidene shows better activity than, the 4-chlorobenzylidene-3-quinolino carbohydrazide substitution (MIC = 0.98, 15.63 µg/mL, respectively). Whilst, changing position of the chloro group from 7- to 6-position resulted in a substantial loss of activity from 0.98 to 7.81 µg/mL, against *Streptococus pneumonia*. Other compounds also showed moderate to good activities against *Streptococus pneumonia* and *Staphylococcus aureus* at different concentrations levels from 1.95 to 3.9 µg/mL.

In general, methylnicotinohydrazone analogs demonstrated better activities over the quinolinyl derivatives which was also greatly enhanced by incorporating electron-donating groups into the benzylidene moiety for example compounds **6l**, **6m**, **6o**, **6p** and **6r**, whereas this activity decreased by the incorporation of electron-withdrawing groups in the following order from 2,5-$(OCH_3)_2$ >3,4,5-$(OCH_3)_2$> 4-Cl > 4-OCH_3 >2,4-$(Cl)_2$> F.

Regarding gram negative bacteria, remarkable activities was elicited by the derivatives **6a**, **6f**, **6p** and **12b** against *Escherichia coli* showing two fold the potency of the standard, ciprofloxacin (MIC = 0.49, 0.98 µg/mL respectively).

In addition, compound **6r** showed very good activity against *Escherichia coli* with MIC = 0.98 as the standard ciprofloxacin, alongside good to moderate activities were also demonstrated by compounds **6e**, **6h**, **6l** and **12e**, finally, it is worth mentioning that substitution on the benzylideine with 4-Floro or 4-methyl abolished the activity.

Unfortunately, all the synthesized compounds were inactive against *Pseudomonas aeruginosa*. Whereas, the results obtained by compounds **6a**, **6f**, **6p**, and **12b** against *Escherichia coli*, were greatly appreciated due to the resistances of gram-negative bacteria [51].

2.2.3. Antimycobacterial Activity

The activity of the target synthesized compounds **6a-l** and **12a-l** were evaluated against *M. tuberculosis* (RCMB 010126) using the microplate Alamar blue assay (MABA) [52]. Isoniazide was used as reference drug. The results of the *in vitro* antimycobacterial screening, as percent inhibition and minimum inhibitory concentration (MIC), are present in **Table 3.**

Table 3.

The results revealed that compounds **6o**, **6p**, and **6r** possessed superior activities against *M. tuberculosis* with MIC = 0.39 µg/mL, 2-folds the activity of isoniazide (MIC = 0.75 µg/mL). Interestingly, these derivatives had electron-donating groups on the benzylidene moiety, 2,5-$(OCH_3)_2$ as in compounds **6p** and **6r** or 2-OH-3-OCH_3 as compound **6o**. In addition, compounds **6e**, **6f**, **6l** and **6n** also showed very good antimycobaterial activities with MIC values 0.78 µg/mL, equipotent to the standard isoniazide (MIC = 0.75 g/mL)..

Other methylnicotinohydrazone derivatives possessed antimycobacterial activity better than INH or equal to it such as **6c**, **6d**, **6k**, **6m** and **6q** at dose levels ranging from 1.56 to 3.12 µg/mL. These results once again, demonstrates the marked effect of electron-donating groups, mostly represented by $–OCH_3$ groups, over the other substituents. Moreover, there was no substantial impact of changing the position of chloro neither in the methylnicotinohydrazone nor in the quinolone series. In general, all the 1,4-dihydroquinoline-3-carbohydrazide derivatives were inactive against *M. tuberculosis*.

In conclusion, the 6-(4-Chlorophenyl)-*N*'-(2,5-dimethoxybenzylidene)-2-methylnicotinohydrazide **6p** emerged as the most potent derivative amongst all the synthesized compounds, exhibiting

promising results over a range of antifungal, antibacterial and antimycobacterial organisms with significant dose levels 0.39 – 0.98 µg/mL, compared to the reference drugs.

2.2.4. In vitro cytotoxic activity:

In addition to the antimycobacterial evaluation, the most active compounds **6d, 6e, 6f, 6k, 6l, 6m, 6n, 6o, 6p ,6q** and **6r** were evaluated for cytotoxic activities against human lung fibroblast normal cells (WI-38), in order to determine the selectivity of hydrazones towards *M.tuberculosis*. Results presented in **(Table 4)** indicate that all the tested hydrazones displayed poor or no cytotoxic activity at 0-500 µg/mL concentrations. Thus compounds **6d, 6e, 6f, 6k, 6l, 6m, 6n, 6o, 6p ,6q** and **6r** showed high selectivity towards *M.tuberculosis.*

Table 4.

2.3. 2D QSAR study:

2.3.1. Development of QSAR models:

With the aim to assess the structural basis for antifungal, antibacterial and antimycobacterial inhibitory activity of the novel derivatives (**6a-r** and **12a-l**), 2D-QSAR analysis was carried out. This analysis was run by means of the DS 4.0 software (Discovery Studio 4.0, Accelrys, Co. Ltd) [53].

A set of the newly synthesized hydrazones (**6a-r** and **12a-l**) was used as training set with their measured pMIC against *Aspergillus fumigatus, Streptococus pneumoniae* and *Mycobacterium tuberculosis* for QSAR modeling, compounds **6f**, **12g** and **12h** were chosen as statistical outliers while building model for *Aspergillus fumigatus,* and *Streptococus pneumoniae* whereas, compounds **6f**, **6j**, **12g** and **12h** were chosen for the *Mycobacterium tuberculosis* model.

"Calculate Molecular Properties" module was used for calculating the molecular properties. Different molecular descriptors were utilized in the models generation representing thermodynamic, electronic, spatial, structural, thermodynamic, geometric, topological and quantum mechanical properties. "Genetic function approximation" (GFA) protocol was applied in order to choose the best descriptors that characterizes the activity. "Multiple linear regression" (MLR) protocol was then employed to search for optimal QSAR models with the best statistical validation measures and capable of correlating bioactivity variation across the used training set collection. QSAR model was validated employing leave one-out cross-validation by setting the folds to a number much larger than the number of samples , r^2 (squared correlation coefficient value) and r^2 prediction (predictive squared correlation coefficient value), residuals between the predicted and experimental activity of the test set and training set.

Equation 1 Represents the best performing QSAR model for the activity against *Aspergillus fumigatus*;

-logMIC= 0.1402 + 0.4747 **Kappa_3_AM** - 4.272 **Shadow_YZfrac**

Equation 2 Represents the best performing QSAR model for the activity against *Streptococus pneumoniae*;

-logMIC= 3.992 + 0.2114 **ALogP** - 8.107 **Shadow_YZfrac**

Equation 3 Represents the best performing QSAR model for *M.tuberculosis*;

-logMIC= 9.377 + 0.8318 **ALogP** - 59.44 **Jurs_RPCG** + 0.01429 **Jurs_WNSA_2**

According to the former equations these QSAR models were represented graphically by scattering plots of the experimental versus the predicted bioactivity values –logMIC for the training set compounds as shown in Figures 2- 4.

The three MLR models exhibited good correlation coefficient **r2** of 0.784, 0.776 and 0.884, **r2 (adj)** = 0.754, 0.744 and 0.862, respectively, **r2 (pred)** = 0.672, 0.673 and 0.803, Least-Squared error = 0.2196, 0.2562 and 0.2201 respectively, where r2 (adj) is r2 adjusted for the number of terms in the model; r2 (pred) is the prediction r2, equivalent to q2 from a leave-1-out cross-validation [54].

Equation 1 suggested that the antifungal activity of the synthesized compounds was positively affected by Kappa_3_AM, which is a topological molecular property describing the molecular shape index. The antifungal and antibacterial activities were also affected by the high and negative value of Shadow_YZfrac. Shadow indices are a set of geometric descriptors that characterize the shape of the molecules. They are calculated by projecting the model surface on three mutually perpendicular planes: xy, yz, and xz. These descriptors depend not only on conformation, but also on the orientation of the model. In order to calculate them, the models are first rotated to align the principal moments of inertia with the x-, y-, and z-axes. Shadow_YZ is area of the molecular shadow in the yz plane [55].

Whereas, equations 2 and 3 showed that, ALogP contributed positively to the antibacterial and antimycobacterial activity. ALogP is a descriptor that computes the lipophilicity of the molecule and is calculated in Discovery Studio as the Log of the octanol water partition coefficient using Ghose and Crippen's method [56]. Interestingly lipophilicity is one of the fundamental molecular properties controlling the antimycobacterial activity [18,57].

Equation 3 showed that the antimycobacterial activities were also affected by jurs descriptors. Jurs descriptors are a group of geometric descriptors, that combine both shape and electronic information which may characterize the molecules [56]. These descriptors are calculated by mapping atomic partial charges on solvent-accessible surface areas of individual atoms. In particular, Jurs_RPCG which contributes inversely to the activity, represents the relative positive charge on the molecule. This indicates that less positive charge on the molecule gives better activity.

2.3.3. QSAR validation

The established QSAR models (1, 2 and 3) were verified by applying; Leave-one-out (LOO) internal validation (**r2** = 0.784, 0.776 and 0.884, respectively). Cross-validation was also employed where **q**2, which is equivalent to **r2 (pred),** was 0.672, 0.673 and 0.803, respectively. In addition, validation was employed by measuring the residuals between the experimental and the predicted activities of the training set listed in (Table 4 and Table 5). Interestingly, the predicted activities of the QSAR models were found very close to those experimentally observed, Furthermore, to evaluate the predictive ability of the developed models *Aspergillus fumigatus*, and *Streptococus pneumoniae*, compounds **6f**, **12g** and **12h** were applied as test compounds, where they were not included in model generation, the same was also applied for the *Mycobacterium tuberculosis* model using compounds **6f**, **6j**, **12g** and **12h**.

Table 5.

Table 6.

2.4. ADME study

The ADME of the biologically active hybrids (**6a-r** and **12a-l**) was predicted via a theoretical kinetic study that is performed by means of Discovery Studio software (Table 6). Both, AlogP98 and PSA_2D descriptors were calculated to evaluate the lipophilicity and polar surface area. Also, solubility, absorption and CYP2D inhibition levels were predicted. Active members of the first series **6a-r** and compound **12h** were found to have good lipophilicity, ALogP values ranging from 2.64 to 5.75, this lipophilicity was found to play an important role in antimycobacterial activity [58]. All series of quinolone compounds **12a-l** showed low lipophilicity levels except 12h. Whilst, all the examined derivatives seemed to possess good absorption levels and predicted to be CYP2D non-inhibitors, generally all the molecules passed the Lipinski's rule of five.

Table 7.

3. Conclusion

In this work two series of nicotinc acid hydrazone derivatives **6a-r** and quinolone hydrazone **12a-l** were synthesized. The antimycobacterial and antimicrobial activity of the newly synthesized target compounds were evaluated. The results manifested that compounds **6p** and **6f** show broad spectrum of activity against antifungal, gram positive and gram negative bacteria. In addition, the methylnicotinohydrazone derivatives **6o**, **6p** and **6r** showed the most promising antimmycobacterial activity against *M. tuberculosis* with MIC = 0.39 µg/mL. Moreover, compounds **6e**, **6f**, **6l** and **6n** also showed good antimycobaterial activities with MIC values 0.78 µg/mL. These results manifested that 6-(4-chlorophenyl)-*N*'-(2,5-dimethoxybenzylidene)-2-methylnicotinohydrazide **6p** could be further investigation as apromising candidate for a potential antimicrobial agent. Finally, the generated QSAR models, performed to explore the structural requirements controlling the observed antibacterial properties, indicated that the antibacterial and antimmycobacterial were affected by lipophilicity and that these generated models had good prediction ability.

4. Experimental

4.1. Chemistry

4.1.1. General

Melting points were measured with a Stuart melting point apparatus and were uncorrected. The NMR spectra were recorded by Varian Gemini-400BB 400 MHz FT-NMR spectrometers (Varian Inc., Palo Alto, CA). ^{1}H and ^{13}C spectra were run at 400 and 100 MHz, respectively, in deuterated dimethylsulphoxide (DMSO-d_6). Chemical shifts (δ_H) are reported relative to TMS as internal standard. All coupling constant (J) values are given in hertz. Chemical shifts (δ_C) are reported relative to DMSO-d_6 as internal standards. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. IR spectra were recorded with a Bruker FT-IR spectrophotometer. Reaction courses and product mixtures were routinely monitored by thin layer chromatography (TLC) on silica gel precoated F_{254} Merck plates. Unless otherwise noted, all solvents and reagents were commercially available and used without further purification.

4.1.2. Ethyl 2-methyl-6-arylnicotinate **3a,b**.

To a solution of the appropriate enaminone **2a,b** (5 mmol) in glacial acetic acid (15 mL), ethyl acetoacetate (5.5 mmol) and ammonium acetate (40 mmol) were added. The reaction mixture was heated under reflux for 5 h. After cooling and pouring into ice-water, the residue obtained was filtered and washed with petroleum ether then with water and finally crystallized from ethanol [59].

4.1.2.1. 6-(3-Chlorophenyl)-2-methylnicotinate ester (**3a**).

White crystals (yield 80%), m.p. 62-65°C; IR (KBr, ν cm^{-1}): 1716 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 1.34 (t, 3H, C<u>H</u>$_3$), 2.50 (s, 3H, CH_3 pyridine), 4.31 (q, 2H, C<u>H</u>$_2$ –CH_3), 7.54 (d, 2H, H-4, H-6, 3-Cl-C_6H_4), 7.79 (d, 1H, *J* = 8.0 Hz, H-4 pyridine,), 8.09-8.11 (m, 1H,H-5, 3-Cl-C_6H_4), 8.19 (s, 1H, H-2, 3-Cl-C_6H_4), 8.24 (d, 1H, *J* = 8.0 Hz, H-5 pyridine) ; ^{13}C NMR (DMSO-d_6) δ *ppm*: 14.51, 25.10, 61.50 (CH_3), 118.32, 124.71, 126.03, 127.06, 130.11, 131.21, 134.28, 139.89, 140.01, 156.41, 159.07, 166.24.

4.1.2.2. 6-(4-Chlorophenyl)-2-methylnicotinate ester (**3b**).

White crystals (yield 75%), m.p. 42-47°C, as reported [60].

4.1.3. 6-Aryl-2-methylnicotinohydrazide **4a,b**.

A mixture of the appropriate ester **3a,b** (5 mmol) and 99% hydrazine hydrate (2 mL) was refluxed for 6 h. The solid product obtained upon cooling was filtered off and recrystallized from dioxan to afford the corresponding 6-aryl-2-methylnicotinohydrazides **4a,b**. [59].

4.1.3.1. 6-(3-Chlorophenyl)-2-methylnicotinohydrazide (**4a**).

White crystals (yield 90%), m.p. 208-210 °C; IR (KBr, ν cm^{-1}): 3282, 3321 (NH, NH_2) and 1660 (C=O); ^{1}H NMR (DMSO-*d6*) δ *ppm*: 2.61 (s, 3H, CH_3 pyridine), 4.54 (s, 2H, NH_2, D_2O exchangeable), 7.50-7.55 (m, 2H, H-4, H-6, 3-Cl-C_6H_4), 7.78 (d, 1H, *J* = 8.0 Hz, H-4 pyridine,), 7.92 (d, 1H, *J* = 8.0 Hz, H-5 pyridine), 8.06-8.09 (m, 1H, H-5, 3-Cl-C_6H_4), 8.16 (s, 1H, H-2, 3-Cl-C_6H_4), 9.63 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 23.04, (CH_3), 117.87, 125.69, 126.73, 129.57, 130.29, 131.16, 134.21, 137.02, 140.54, 154.50, 156.22, 167.43.

4.1.3.2. 6-(4-Chlorophenyl)-2-methylnicotinohydrazide (**4b**).

White crystals (yield 75%), m.p. 215-220 °C, as reported [60].

4.1.4.1. Preparation of 6-(3-Chlorophenyl)-*N*'-(4-chlorobenzylidene)-2-methylnicotinohydrazide (**6a**).

Different aldehydes **5a-j**, (1 mmol) was added to a suspension of 6-aryl-2-methylnicotinohydrazide derivatives **4a, b** (1 mmol) in ethanol (10 mL) and catalytic amount of glacial acetic acid. The reaction mixture was refluxed for 4 h. The precipitate formed was collected by filtration while hot, washed with hot ethanol, dried and crystallized from ethanol to furnish compounds **6a-r**.

White crystals (yield 90%), m.p. 215-220 °C; IR (KBr, ν cm^{-1}): 3174 (NH) and 1651 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.50, 2.63 (s, 3H, CH_3), 7.40- 7.42 (m, 2H, H-3, H-5, 4-Cl-C_6H_4), 7.51-7.55 (m, 2H, H-4, H-6, 3-Cl-C_6H_4), 7.74 (d, 2H, *J* = 8.0 Hz, H-2, H-6, 4-Cl-C_6H_4), 7.82 (d, *J* = 8.0 Hz, 1H, H-4 pyridine), 7.93 (d, 1H, *J* = 8.0 Hz, H-5 pyridine), 8.07-8.11 (m, 1H, H-5, 3-Cl-C_6H_4), 8.17 (s, 1H, H-2, 3-Cl-C_6H_4), 8.28, 8.00 (s, 1H, C<u>H</u>=N), 11.98, 12.04 (s, 1H, –CONH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 23.37, 23.43, (CH_3), 117.63, 117.96, 125.74, 125,83, 126.81, 126.84, 128.72, 129.27, 129.43, 129.59, 129.75, 129.85, 130.68, 131.20, 133.25, 133.52, 134.26, 134.81, 135.15, 137.40, 137.74, 140.44, 140.58, 143.72, 147.09, 154.29, 155.03, 156.51, 164.34, 170.35; Anal. calcd. for $C_{20}H_{15}Cl_2N_3O$ (384.26): C, 62.51; H, 3.93; N, 10.94. Found C, 63.04; H, 4.03; N, 11.35.

4.1.4.2. 6-(3-Chlorophenyl)-*N*'-(2,4-dichlorobenzylidene)-2-methylnicotinohydrazide (**6b**).

White crystal (yield 80%), m.p. 245-250 °C; IR (KBr, ν cm^{-1}): 3170 (NH) and 1662 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.51, 2.65 (s, 3H, CH_3), 7.52- 7.55 (m, 2H, H-4, H-6, 3-Cl-C_6H_4), 7.64, 7.71 (s, 1H, H-3, 2,4$(Cl)_2$-C_6H_3), 7.84, 7.38 (d, 1H, *J* = 8.4 Hz, H-4 pyridine), 7.92, 7.34 (d, 1H, *J* = 8.0 Hz, H-5 pyridine), 7.97- 8.01 (m, 2H, H-5, 3-Cl-C_6H_4, and H-5, 2,4$(Cl)_2$-C_6H_3), 8.09 (d, 1H, H-6, 2,4$(Cl)_2$-C_6H_3), 8.18, 8.04 (s, 1H, H-2, 3-Cl-C_6H_4), 8.43, 8.66 (s, 1H, C<u>H</u>=N), 12.15, 12.36 (s, 1H, –CONH, D_2O exchangeable); Anal. calcd. for $C_{20}H_{14}Cl_3N_3O$ (418.70): C, 57.37; H, 3.37; N, 10.04. Found C, 57.71; H, 3.39; N, 10.57.

4.1.4.3. 6-(3-Chlorophenyl)-*N*'-(4-florobenzylidene)-2-methylnicotinohydrazide (**6c**).

White crystals (yield 60%), m.p. 180-183 °C; IR (KBr, ν cm^{-1}): 3178 (NH) and 1643 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.50, 2.63 (s, 3H, CH_3), 7.27, 7.14 (t, 2H, *J* = 8.8 Hz, H-3, H-5, 4-F-C_6H_4), 7.50-7.55 (m, 2H, H-4, H-6, 3-Cl-C_6H_4), 7.77-7.80 (dd, 2H, *J* = 6.0 Hz, *J* = 8.8 Hz, H-2 and H-6, 4-F-C_6H_4), 7.82, 7.99 (d, 1H, *J* = 8.0 Hz, H-4 pyridine), 7.93 (d, 1H, *J* = 8.0 Hz, H-5 pyridine), 8.08- 8.10 (m, 1H, H-5, 3-Cl-C_6H_4), 8.17 (s, 1H, H-2, 3-Cl-C_6H_4) 8.29 (s, 1H, C<u>H</u>=N), 11.91, 11.97 (s, 1H, –CONH, D_2O exchangeable); MS *m/z* %: 369.06 (M^++2, 0.97), 367.09 (M^+, 2.30), 230.05 (100%); Anal. calcd. for $C_{20}H_{14}ClFN_3O$ (367.81): C, 65.31; H, 4.11; N, 11.42. Found C, 65.48; H, 4.18; N, 11.80.

4.1.4.4. 6-(3-Chlorophenyl)-*N*'-(4-methylbenzylidene)-2-methylnicotinohydrazide (**6d**).

White crystals (yield 70%), m.p. 210-213 °C; IR (KBr, ν cm^{-1}): 3213 (NH) and 1651 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.24, 2.33 (s, 3H, CH_3 C_6H_5), 2.50, 2.63 (s, 3H, CH_3), 7.12, 7.25 (d, 2H, *J* = 8.0 Hz, H-3, H-5, 4-CH_3-C_6H_4), 7.50-7.53 (m, 2H, H-4, H-6, 3-Cl-C_6H_5), 7.55, 7.60 (d, 2H, *J* = 8.0 Hz, H-2, H-6, 4-CH3-C_6H_4), 7.82, 7.97 (d, 1H, *J* = 8.4 Hz, H-4 pyridine), 7.93, 7.95 (d, 1H, *J* =

5.6 Hz, H-5 pyridine), 8.08-8.10 (m, 1H, H-5, 3-Cl-C_6H_4), 8.17, (s, 1H, H-2, 3-Cl-C_6H_4), 8.25, 8.05 (s, 1H, C<u>H</u>=N), 11.84, 11.90 (s, 1H, –CONH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 21.43, 21.50, 23.41, (CH_3), 117.61, 117.95, 125.74, 125,81, 126.83, 127.08, 127.62, 129.56, 129.72, 129.87, 129.94, 130.04, 131.16, 131.20, 134.25, 137.36, 137.46, 140.47, 140.57, 145.01, 148.44, 154.93, 156.46, 164.20, 170.17; MS *m/z* %: 365.12 (M^++2, 1.48), 363.10 (M^+, 3.79), 230.06 (100%); Anal. calcd. for $C_{21}H_{18}ClN_3O$ (363.85): C, 69.32; H, 4.99; N, 11.55. Found C, 69.55; H, 5.04; N, 11.78.

4.1.4.5. 6-(3-Chlorophenyl)-*N*′-(3,4-dimethoxybenzylidene)-2-methylnicotinohydrazide (**6e**).

White crystal (yield 75%), m.p. 190-192 °C; IR (KBr, ν cm^{-1}): 3221 (NH) and 1651 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.51, 2.63 (s, 3H, CH_3), 3.56, 3.79 (s, 3H, OCH_3-C_6H_3), 3.70, 3.81 (s, 3H, OCH_3-C_6H_3), 7.00, 6.89 (d, 1H, *J* = 8.0 Hz, H-5, 3,4-$(OCH_3)_2$-C_6H_3), 7.18, 6.96 (d, 1H, *J* = 8.4 Hz, H-6, 3,4-$(OCH_3)_2$-C_6H_3), 7.33, 6.92 (s, 1H, H-2, 3,4-$(OCH_3)_2$-C_6H_3), 7.52-7.55 (m, 2H, H-4, H-6, 3-Cl-C_6H_5), 7.82 (d, 1H, H-4 pyridine), 7.93 (d, 1H, *J* = 8.0 Hz, H-5 pyridine), 8.06- 8.09 (m, 1H, H-5, 3-Cl-C_6H_4), 8.17 (s, 1H, H-2, 3-Cl-C_6H_5), 8.21, 7.98 (s, 1H, C<u>H</u>=N), 11.78, 12.07 (s, 1H, –CONH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 23.39, (CH_3), 55.91, 56.03, (OCH_3), 108.71, 111.93, 117.96, 122.46, 125.80, 126.82, 127.22, 129.70, 130.15, 131.20, 134.25, 137.33, 140.48, 148.55, 149.53, 154.90, 156.41, 164.09; Anal. calcd. for $C_{22}H_{20}ClN_3O_3$ (409.87): C, 64.47; H, 4.92; N, 10.25. Found C, 64.73; H, 4.97; N, 10.49.

4.1.4.6. 6-(3-Chlorophenyl)-*N*′-(3,4,5-trimethoxybenzylidene)-2-methylnicotinohydrazide (**6f**).

White crystal (yield 80%), m.p. 200-203 °C; IR (KBr, ν cm^{-1}): 3224 (NH) and 1651 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.51, 2.64 (s, 3H, CH_3), 3.61 (s, 3H, OCH_3-C_6H_3), 3.70 (s, 3H, OCH_3-C_6H_3), 3.82 (s, 3H, OCH_3-C_6H_3), 7.02, 6.7 (s, 2H, H-2, H-6, 3,4,5-$(OCH_3)_3$-C_6H_2), 7.51- 7.55 (m, 2H, H-4, H-6, 3-Cl-C_6H_4), 7.83 (d, 1H, H-4 pyridine), 7.92 (d, 1H, H-5 pyridine), 8.04- 8.09 (m, 1H, H-5, 3-Cl C_6H_4), 8.17 (s, 1H, H-2, 3-Cl C_6H_4), 8.22, 8.13 (s, 1H, C<u>H</u>=N), 11.87, 12.00 (s, 1H, –CONH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 23.39, (CH_3), 56.04, 56.42, 60.57, (OCH_3), 104.84, 117.99, 125.82, 126.83, 129.72, 130.04, 130.06, 131.21, 134.26, 137.38, 139.77, 140.47, 148.35, 153.65, 154.97, 156.41, 164.25; Anal. calcd. for $C_{23}H_{22}ClN_3O_4$ (439.90): C, 62.80; H, 5.04; N, 9.55. Found C, 62.80; H, 5.08; N, 9.95.

4.1.4.7. 6-(4-Chlorophenyl)-*N*′-(4-chlorobenzylidene)-2-methylnicotinohydrazide (**6g**).

White crystals (yield 90%), m.p. 215-220 °C; IR (KBr, ν cm^{-1}): 3228 (NH) and 1654 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.94, 2.62 (s, 3H, CH_3), 7.40- 7.42 (m, 2H, H-3, H-5, 4-Cl-C_6H_4 (ald)), 7.51- 7.85 (m, 2H, , H-3, H-5, 4-Cl C_6H_4), 7.74 (d, 2H, *J* = 8.0 Hz, H-2, H-6, 4-Cl C_6H_4 (ald)),

7.81, 7.93 (d, 1H, *J* = 8 Hz, H-4 pyridine), 7.89, 7.95 (d, 1H, *J* = 8 Hz, H-5 pyridine), 7.95-7.97 (m, 2H, H-2 and H-6, 4-Cl-C_6H_4), 8.27, 8.07 (s, 1H, C<u>H</u>=N), 11.98, 12.05 (s, 1H, –CONH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 23.44, (CH_3), 117.59, 123.85, 128.70, 128.98, 129.26, 129.30, 129.42, 129.48, 133.51, 134.84, 137.13, 137.35, 147.04, 155.42, 156.43, 164.40, 168.14; Anal. calcd. for $C_{20}H_{15}Cl_2N_3O$ (384.26): C, 62.51; H, 3.93; N, 10.94. Found C, 62.74; H, 3.97; N, 11.47.

4.1.4.8. 6-(4-Chlorophenyl)-*N*'-(2,4-dichlorobenzylidene)-2-methylnicotinohydrazide (**6h**).

White crystal (yield 80%), m.p. 245-250 °C; IR (KBr, ν cm^{-1}): 3170 (NH) and 1665 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.50, 2.64 (s, 3H, CH_3), 7.54 (d, 2H, *J* = 8.8 Hz, H-3, H-5, 4-Cl-C_6H_4), 7.64, 7.71 (s, 1H, H-3, 2,4$(Cl)_2$-C_6H_3), 7.83, 7.38 (d, 1H, H-4 pyridine), 7.88, 7.34 (d, 1H, H-5 pyridine), 7.93 (d, 1H, *J* = 8.4 Hz, H-6, 2,4$(Cl)_2$-C_6H_3), 7.99- 8.03 (m, 1H, H-5, 2,4$(Cl)_2$-C_6H_3), 8.15 (d, 2H, *J* = 8.8 Hz, H-2, H-6, 4-Cl-C_6H_4), 8.43, 8.66 (s, 1H, C<u>H</u>=N), 12.14, 12.17 (s, 1H, –CONH, D_2O exchangeable); Anal. calcd. for $C_{20}H_{14}Cl_3N_3O$ (418.70): C, 57.37; H, 3.37; N, 10.04. Found C, 57.98; H, 3.39; N, 10.35.

4.1.4.9. 6-(4-Chlorophenyl)-*N*'-(4-florobenzylidene)-2-methylnicotinohydrazide (**6i**).

White crystals (yield 60%), m.p. 180-183 °C; IR (KBr, ν cm^{-1}): 3167 (NH) and 1643 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.50, 2.63 (s, 3H, CH_3), 7.26, 7.14 (t, 2H, H-3, H-5, 4-F-C_6H_4), 7.54 (d, 2H, *J* = 8.5 Hz, H-3, H-5, 4-Cl-C_6H_4), 7.76, 7.42 (dd, 2H, *J* = 5.6 Hz, *J* = 8.8 Hz, H-2 and H-6, 4-F-C_6H_4), 7.81, 7.91 (d, 1H, *J* = 8.4 Hz, H-4 pyridine), 7.88, 7.94 (d, 1H, *J* = 8.0 Hz, H-5 pyridine), 8.14 (d, *J* = 8.4 Hz, 2H, H-2, H-6, 4-Cl-C_6H_4), 8.30, 8.08 (s, 1H, C<u>H</u>=N), 11.87, 11.94 (s, 1H, –CONH, D_2O exchangeable); Anal. calcd. for $C_{20}H_{14}ClFN_3O$ (367.81): C, 65.31; H, 4.11; N, 11.42. Found C, 65.59; H, 4.18; N, 11.93.

4.1.4.10. 6-(4-Chlorophenyl)-*N*'-(4-methylbenzylidene)-2-methylnicotinohydrazide (**6j**).

White crystals (yield 70%), m.p. 210-213 °C; IR (KBr, ν cm^{-1}): 3163 (NH) and 1643 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.24, 2.33 (s, 3H, CH_3-C_6H_4), 2.49, 2.63 (s, 3H, CH_3), 7.12, 7.25 (d, 2H, *J* = 8.0 Hz, H-3, H-5 of 4-CH_3-C_6H_4), 7.54 (d, 2H, H-3, H-5, 4-Cl-C_6H_4), 7.60 (d, 2H, *J* = 8.0 Hz, H-2, H-6 of 4-CH_3-C_6H_4), 7.80, 7.88 (d, 1H, *J* = 7.6 Hz, H-4 pyridine), 7.91, 7.93 (d, *J* = 8.4 Hz, 1H, H-5 pyridine), 8.14 (d, 2H, H-2, H-6, 4-Cl-C_6H_4), 8.25, 8.05 (s, 1H, C<u>H</u>=N), 11.81, 11.88 (s, 1H, –CONH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 21.50, 23.43, (CH_3), 117.58, 127.08, 127.60, 128.92, 128.97, 129.26, 129.30, 129.87, 129.94, 131.85, 137.17, 137.33, 140.55, 148.39, 155.34, 156.40, 164.25; Anal. calcd. for $C_{21}H_{18}ClN_3O$ (363.85): C, 69.32; H, 4.99; N, 11.55. Found C, 69.45; H, 5.09; N, 11.97.

4.1.4.11. 6-(4-Chlorophenyl)-*N*'-(3,4-dimethoxybenzylidene)-2-methylnicotinohydrazide (**6k**).

White crystal (yield 75%), m.p. 190-192 °C; IR (KBr, ν cm^{-1}): 3190 (NH) and 1651 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.50, 2.62 (s, 3H, CH_3), 3.56, 3.79 (s, 3H, OCH_3-C_6H_3), 3.70, 3.81 (s, 3H, OCH_3-C_6H_3), 7.00, 6.89 (d, 1H, *J* = 8.0 Hz, H-5, 3,4-$(OCH_3)_2$-C_6H_3), 7.18, 6.96 (d, 1H, *J* = 8.4 Hz, H-6, 3,4-$(OCH_3)_2$-C_6H_3), 7.33, 6.92 (s, 1H, H-2, 3,4-$(OCH_3)_2$-C_6H_3), 7.54 (d, 2H, *J* = 8.8 Hz, H-3, H-5, 4-Cl-C_6H_5), 7.81 (d, 1H, *J* = 8.0 Hz, H-4 pyridine), 7.87 (d, 1H, *J* = 8.0 Hz, H-5 pyridine), 8.14 (d, 2H, *J* = 8.8 Hz, H-2, H-6, 4-Cl-C_6H_4), 8.21, 7.97 (s, 1H, C<u>H</u>=N), 11.75, 11.86 (s, 1H, –CONH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 23.41, (CH_3), 55.91, 56.03, (OCH_3), 108.71, 111.93, 117.60, 122.44, 127.23, 128.82, 128.97, 129.30, 129.80, 134.80, 137.20, 137.29, 148.51, 149.53, 151.32, 155.30, 156.35, 164.15; MS *m/z* %: 411.09 (M^++2, 7.49), 409.10 (M^+, 20.34), 230.05 (100%); Anal. calcd. for $C_{22}H_{20}ClN_3O_3$ (409.87): C, 64.47; H, 4.92; N, 10.25. Found C, 64.72; H, 4.92; N, 10.64.

4.1.4.12. 6-(4-Chlorophenyl)-*N*'-(3,4,5-trimethoxybenzylidene)-2-methylnicotinohydrazide (**6l**).

White crystal (yield 80%), m.p. 200-203 °C; IR (KBr, ν cm^{-1}): 3221 (NH) and 1651 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.50, 2.63 (s, 3H, CH_3), 3.61, 3.74 (s, 3H, OCH_3 C_6H_3), 3.69, 3.72 (s, 3H, OCH_3-C_6H_3), 3.82, 3.84 (s, 3H, OCH_3-C_6H_3), 6.70, 7.02 (s, 2H, H-2, H-6, 3,4,5-$(OCH_3)_3$-C_6H_2), 7.54 (d, 2H, *J* = 8.8 Hz, H-3, H-5, 4-Cl-C_6H_5) 7.82 (d, 1H, *J* = 8.0 Hz, H-4 pyridine), 7.87 (d, 1H, *J* = 8.0 Hz, H-5 pyridine), 8.14 (d, 2H, *J* = 8.8 Hz, H-2, H-6, 4-Cl-C_6H_4), 8.10, 8.21 (s, 1H, C<u>H</u>=N), 11.87, 12.00 (s, 1H, –CONH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 23.41, (CH_3), 56.04, 56.42, 56.52, 60.57, (OCH_3), 104.83, 117.62, 128.98, 129.31, 129.71, 130.04, 134.83, 137.18, 137.34, 140.32, 153.65, 155.38, 156.35, 164.31; MS *m/z* %: 441.16 (M^++2, 15.42), 439.17 (M^+, 48.65), 230.06 (100%); Anal. calcd. for $C_{23}H_{22}ClN_3O_4$ (439.90): C, 62.80; H, 5.04; N, 9.55. Found C, 62.98; H, 5.18; N, 9.95.

4.1.4.13. 6-(4-Chlorophenyl)-*N*'-(4-hydroxybenzylidene)-2-methylnicotinohydrazide (**6m**).

White crystals (yield 90%), m.p. 210-215 °C; IR (KBr, ν cm^{-1}): 3170 (NH) and 1662 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.51, 2.66 (s, 3H, CH_3), 6.73, 6.86 (d, 2H, *J* = 8.0 Hz, H-3, H-5, 4-OH-C_6H_4), 7.26, 7.94 (d, 2H, *J* = 8.4 Hz, H-2, H-6, 4-OH C_6H_4), 7.56 (d, 2H, *J* = 8.0 Hz, H-3 and H-5, 4-Cl-C_6H_4), 8.16 (d, 2H, *J* = 8.0 Hz, H-2 and H-6, 4-Cl-C_6H_4), 7.83, 7.96 (d, 1H, *J* = 8.0 Hz, H-4 pyridine), 7.89 (d, 1H, H-5 pyridine), 8.03, 8.27 (s, 1H, C<u>H</u>=N), 9.95 (s, 1H, OH, D_2O exchangeable), 11.73, 11.80 (s, 1H, –CONH, D_2O exchangeable); Anal. calcd. for $C_{20}H_{16}ClN_3O_3$ (365.82): C, 65.67; H, 4.41; N, 11.49. Found C, 62.77; H, 4.55; N, 11.67.

4.1.4.14. 6-(4-Chlorophenyl)-*N'*-(4-methoxybenzylidene)-2-methylnicotinohydrazide (**6n**).

White crystals (yield 80%), m.p. 205-210 °C; IR (KBr, ν cm^{-1}): 3228 (NH) and 1651 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.51, 2.66 (s, 3H, CH_3), 3.73, 3.87 (s, 3H, CH_3 -OCH_3), 6.90, 7.03 (d, 2H, *J* = 8.4 Hz, H-3, H-5, 4-OCH_3- C_6H_4), 7.57 (d, 2H, *J* = 8.4 Hz, H-3, H-5, 4-Cl C_6H_4), 7.69, 7.35 (d, 2H, *J* = 8.4 Hz, H-2, H-6, 4-OCH_3- C_6H_4), 7.83, 7.96 (d, 1H, *J* = 8.0 Hz, H-4 pyridine), 7.89, 7.93 (d, 1H, H-5 pyridine), 8.16 (d, 2H, *J* = 8.4 Hz, H-2, H-6, 4-Cl-C_6H_4), 8.06, 8.27 (s, 1H, C<u>H</u>=N), 11.80, 11.87 (s, 1H, –CONH, D_2O exchangeable); Anal. calcd. for $C_{21}H_{18}ClN_3O_2$ (397.84): C, 66.40; H, 4.78; N, 11.06. Found C, 66.65; H, 4.90; N, 11.14.

4.1.4.15. 6-(4-Chlorophenyl)-*N'*-(2-hydroxy-3-methoxybenzylidene)-2-methylnicotinohydrazide (**6o**).

White crystals (yield 75%), m.p. 200-205 °C; IR (KBr, ν cm^{-1}): 3228 (NH) and 1654 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.48, 2.65 (s, 3H, CH_3), 3.72, 3.80 (s, 3H, CH_3 -OCH_3), 6.67, 6.83 (t, 1H, H-5, 2-OH-3-OCH_3-C_6H_3), 6.89 (dd, 1H, *J* = 1.2 Hz, *J* = 8.0 Hz, H-4, 2-OH-3-OCH_3-C_6H_3), 7.55, 7.02 (d, 1H, *J* = 8.0 Hz, H-6, 2-OH-3-OCH_3-C_6H_3), 7.55 (d, 2H, *J* = 8.4 Hz, H-3, H-5, 4-Cl-C_6H_4), 7.82, 7.99 (d, 1H, *J* = 8.0 Hz, H-4 pyridine), 7.90, 7.93 (d, 1H, *J* = 8.4 Hz, H-5 pyridine), 8.15 (d, 2H, *J* = 8.4 Hz, H-2, H-6 of 4-Cl C_6H_4), 8.36, 8.52 (s, 1H, C<u>H</u>=N), 10.70 (s, 1H, OH, D_2O exchangeable), 12.06 (s, 1H, –CONH, D_2O exchangeable); Anal. calcd. for $C_{21}H_{18}ClN_3O_3$ (395.84): C, 63.72; H, 4.58; N, 10.62. Found C, 63.95; H, 4.76; N, 10.85.

4.1.4.16. 6-(4-Chlorophenyl)-*N'*-(2,5-dimethoxybenzylidene)-2-methylnicotinohydrazide (**6p**).

White crystals (yield 85%), m.p. 220-225 °C; IR (KBr, ν cm^{-1}): 3178 (NH) and 1604 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.51, 2.67 (s, 3H, CH_3), 3.78, 3.81 (s, 6H, CH_3 -OCH_3), 6.90, 7.07 (m, 2H, H-3, H-4, 2,5-$(OCH_3)_2$-C_6H_3), 7.41, 6.81 (s, 1H, H-6, 2,5-$(OCH_3)_2$-C_6H_3), 7.56 (d, 1H, *J* = 8.0 Hz, H-3, H-5, 4-Cl-C_6H_4), 7.84, 7.99 (d, 1H, *J* = 8.0 Hz, H-4 pyridine), 7.90, 7.93 (d, 1H, *J* = 8.0 Hz, H-5 pyridine), 8.17 (d, 1H, *J* = 8.0 Hz, H-2, H-6, 4-Cl-C_6H_4), 8.39, 8.67 (s, 1H, C<u>H</u>=N), 11.93, 11.98 (s, 1H, –CONH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 23.44, (CH_3), 55.45, 55.94, 56.63. 56.72, (OCH_3), 109.60, 109.96, 113.71, 113.93, 117.00. 117.10, 117.55, 118.39, 123.15, 128.84, 128.98, 129.29, 129.50, 130.55, 134.64, 134.85, 137.19, 137.36, 139.86, 143.81, 152.52, 152.85, 153.43, 153.76, 154.67, 155.07, 155.40, 156.57, 164.25, 170.36; Anal. calcd. for $C_{22}H_{20}ClN_3O_3$ (409.87): C, 64.47; H, 4.92; N, 10.25. Found C, 64.72; H, 4.92; N, 10.64.

4.1.4.17. 6-(3-Chlorophenyl)-*N'*-(4-methoxybenzylidene)-2-methylnicotinohydrazide (**6q**).

White crystals (yield 90%), m.p. 203-208 °C; IR (KBr, ν cm^{-1}): 3178 (NH) and 1604 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.51, 2.67 (s, 3H, CH_3), 3.74, 3.83 (s, 3H, CH_3 -OCH_3), 6.90, 7.03 (d, 2H, *J* = 8.0 Hz, H-3, H-5, 4-OCH_3-C_6H_4), 7.55- 7.58 (m, 2H, H-4, H-6, 3-Cl C_6H_4), 7.69, 7.36 (d, 2H, H-2, H-6, 4-OCH_3-C_6H_4), 7.85 (d, 1H, H-4 pyridine), 7.95 (d, 1H, H-5 pyridine), 8.12- 8.22 (m, 2H, H-2, H-5, 3-Cl C_6H_4), 8.07, 8.28 (s, 1H, C<u>H</u>=N), 11.83, 11.86 (s, 1H, –CONH, D_2O exchangeable); Anal. calcd. for $C_{21}H_{18}ClN_3O_2$ (379.84): C, 66.40; H, 4.78; N, 11.06. Found C, 66.65; H, 4.90; N, 11.14.

4.1.4.18. 6-(3-Chlorophenyl)-*N*'-(2,5-dimethoxybenzylidene)-2-methylnicotinohydrazide (**6r**).

White crystals (yield 90%), m.p. 220-225 °C; IR (KBr, ν cm^{-1}): 3170 (NH) and 1662 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.51, 2.68 (s, 3H, CH_3), 3.78, 3.81 (s, 6H, CH_3 -OCH_3), 6.91- 7.08 (m, 2H, H-3, H-4, 2,5-$(OCH_3)_2$-C_6H_3), 7.42, 6.81 (s, 1H, H-6, 2,5-$(OCH_3)_2$-C_6H_3), 7.54- 7.76 (m, 1H, H-4, H-6, 3-Cl C_6H_4), 7.86 (d, 1H, H-4 pyridine), 7.95 (d, 1H, H-5 pyridine), 8.00- 8.21 (m, 2H, H-2, H-5, 3-Cl C_6H_4), 8.39, 8.67 (s, 1H, C<u>H</u>=N), 11.93, 11.96 (s, 1H, –CONH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 23.44, (CH_3), 55.45, 55.95, 56.64. 56.73, (OCH_3), 109.59, 109.98, 113.72, 113.94, 117.00. 117.49, 117.92, 118.41, 123.14, 125.67, 125.83, 126.76, 126.87, 129.53, 129.72, 129.86, 130.93, 131.17, 134.28, 137.41, 139.92, 140.49, 140.68, 143.85, 152.53, 152.86, 153.43, 153.76, 154.27, 155.07, 155.12, 156.62, 164.19, 170.30; Anal. calcd. for $C_{22}H_{20}ClN_3O_3$ (409.87): C, 64.47; H, 4.92; N, 10.25. Found C, 64.72; H, 4.92; N, 10.64.

4.1.5. Preparation of 1,4-dihydro-4-oxo-quinoline-3-carbohydrazide derivatives **10a**,**b**

Substituted aniline **7a**,**b** (0.01 mol) and diethyl ethoxy methylene malonate (0.01 mol) were mixed and heated at 125-130 ^{0}C for 3 h. Ethanol was evaporated off from the resulting mixture of ethyl anilinomethylene malonate. The crude solid was filtered on sintered funnel, dried. The malonate **8a**,**b** (0.01 mol) was refluxed with diphenylether (50 mL) for 2 h to give 1,4-dihydro-4-oxoquinoline-3-carboxylic acid ethyl ester **9a**,**b**. After 1 h the solution was cooled and the resulting precipitate was filtered off and dried.

Substituted-1,4-dihydro-4-oxoquinoline- 3-carboxylic acid ethyl ester **9a**,**b** (0.01 mol) was refluxed for 12 h with hydrazine hydrate (0.01 mol) in absolute ethanol (9 mL) to give substituted- 4-oxo-1,4 dihydroquinoline-3-carbohydrazide **10a,b**. The excess solvent was evaporated off and the resulting mixture was poured into crushed ice. The solid separated was filter on sintered funnel, washed with water and dried.

The physical properties and spectral data of **10a,b** were being identical with those were reported [61].

4.1.5.1. Preparation of 7-Chloro-*N*'-(4-chlorobenzylidene)-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (**12a**).

1,4-dihydro-4-oxo-quinoline-3-carbohydrazide derivatives **10a,b** (1 mmol) reacted with different aldehydes **11a-f**, (1 mmol) in DMF (10 mL). The reaction mixture was refluxed for 4 h. Then, the precipitate formed was collected by filtration while hot, washed with hot ethanol, dried and crystallized from ethanol\DMF to furnish compounds **12a-l**.

White crystal (yield 86%), m.p. above 300 °C; IR (KBr, ν cm^{-1}): 3251 (NH, NH) and 1666, 1670 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 7.51-7.56 (m, 3H, H-3, H-5, 4-Cl-C_6H_4 and H-6 quinoline), 7.76- 7.79, (m, 3H, H-2, H-6, 4-Cl-C_6H_4 and H-8 quinoline), 8.26 (d, 1H, *J* = 8.8 Hz, H-5 quinoline), 8.44 (s, 1H, C<u>H</u>=N), 8.90 (s, 1H, H-2 quinoline), 13.16 (s, 1H, –CONH, D_2O exchangeable), 13.19 (s, 1H, NH, D_2O exchangeable); Anal. calcd. for $C_{17}H_{11}Cl_2N_3O_2$ (360.18): C, 56.69; H, 3.08; N, 11.67. Found C, 56.69; H, 3.12; N, 11.67.

4.1.5.2. 7-Chloro-*N*'-(2,4-dichlorobenzylidene)-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (**12b**).

White crystal (yield 85%), m.p. above 300 °C; IR (KBr, ν cm^{-1}): 3402 (NH, NH) and 1651, 1660 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 7.45- 7.47 (m, 2H, H-5, 2,4$(Cl)_2$-C_6H_3 and H-6 quinoline), 7.60 (s,1H, H-8 quinoline), 7.68 (s, 1H, H-3, 2,4$(Cl)_2$-C_6H_3), 7.91 (d, 1H, H-6 2,4$(Cl)_2$-C_6H_3), 8.17 (d, 1H, *J* = 8.0 Hz, H-5 quinoline), 8.52 (s, 1H, C<u>H</u>=N), 8.81 (s, 1H, H-2 quinoline), 13.20 (s, 1H, –CONH, D_2O exchangeable), 13.23 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 110.87, 118.80, 126.17, 128.12, 128.41, 128.63, 129.82, 134.31, 138.56, 142.82, 143.73, 145.79, 151.26, 152.39, 159.94, 161.81, 163.16; MS *m/z* %: 441.16 (M^++2, 15.42), 439.17 (M^+, 48.65), 230.06 (100%);Anal. calcd. for $C_{17}H_{10}Cl_3N_3O_2$ (394.64): C, 51.74; H, 2.55; N, 10.65. Found C, 51.82; H, 2.51; N, 10.65.

4.1.5.3. 7-Chloro-*N*'-(4-florobenzylidene)-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (**12c**).

White crystal (yield 79%), m.p. above 300 °C; IR (KBr, ν cm^{-1}): 3248 (NH, NH) and 1662, 1680 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 7.25 (t, 2H, *J* = 8.8 Hz, H-2, H-6, 4-F-C_6H_4), 7.52- 7.55 (m, 1H, H-6 quinoline), 7.79 (s, 1H, , H-8 quinoline), 7.88 (dd, 2H, *J* = 8.8 Hz, *J* = 5.4 Hz, H-3, H-5, 4-F-C_6H_4), 8.29- 8.31 (m, 2H, H-5 quinoline and C<u>H</u>=N), 8.93 (s, 1H, H-2 quinoline), 12.65 (s, 1H, –CONH, D_2O exchangeable), 13.11 (s, 1H, NH, D_2O exchangeable); MS *m/z* %: 345.11 (M^++2,

1.39), 343.09 (M^+, 4.16), 206.04 (100%); Anal. calcd. for $C_{17}H_{11}ClFN_3O_2$ (343.74): C, 59.40; H, 3.23; N, 12.22. Found C, 59.64; H, 3.28; N, 12.22.

4.1.5.4. 7-Chloro-*N*'-(4-methylbenzylidene)-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (**12d**).

White crystal (yield 83%), m.p. above 300 °C; IR (KBr, ν cm^{-1}): 3201 (NH, NH) and 1651, 1660 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.50 (s, 3H, CH_3), 7.26 (d, *J* = 7.6 Hz, 2H, H-3, H-5, 4- CH_3-C_6H_4), 7.53 (d, *J* = 8.4 Hz, 1H, H-6 quinoline), 7.64, (d, *J* = 7.6 Hz, 2H, H-2, H-6, 4-CH_3- C_6H_4), 7.79 (s, 1H, H-8 quinoline), 8.26 (d, *J* = 8.8 Hz, 1H, H-5 quinoline), 8.37 (s, 1H, C<u>H</u>=N), 8.90 (s, 1H, H-2 quinoline), 12.91 (s, 1H, –CONH, D_2O exchangeable), 13.10 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 111.18, 118.92, 119.24, 125.03, 126.05, 127.65, 128.18, 128.94, 129.83, 132.13, 137.95, 140.31, 140.40, 145.57, 148.27, 161.37, 162.77; MS *m/z* %: 441.16 (M^++2, 15.42), 439.17 (M^+, 48.65), 230.06 (100%);Anal. calcd. for $C_{17}H_{10}Cl_3N_3O_2$ (394.64): C, 51.74; H, 2.55; N, 10.65. Found C, 51.82; H, 2.51; N, 10.65; MS *m/z* %: 341.12 (M^++2, 1.96), 339.09 (M^+, 2.41), 230.00 (100%); Anal. calcd. for $C_{18}H_{14}ClN_3O_2$ (339.78): C, 63.63; H, 4.15; N, 12.37. Found C, 63.90; H, 4.23; N, 12.37.

4.1.5.5. 7-Chloro-*N*'-(3,4-dimethoxybenzylidene)-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (**12e**).

White crystal (yield 80%), m.p. above 300 °C; IR (KBr, ν cm^{-1}): 3201 (NH, NH) and 1670, 1680 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 3.81 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 7.03 (d, 1H, *J* = 8.0 Hz, H-5, 3,4-$(OCH_3)_2$-C_6H_3), 7.26 (d, 1H, H-6 quinoline), 7.37 (s, 1H, H-2, 3,4-$(OCH_3)_2$-C_6H_3), 7.53 (d, 2H, *J* = 8.4 Hz, 1H, H-6, 3,4-$(OCH_3)_2$-C_6H_3), 7.80 (s, 1H, H-8 quinoline), 8.27 (d, *J* = 8.4 Hz, 1H, H-5 quinoline), 8.34 (s, 1H, C<u>H</u>=N), 8.89 (s, 1H, H-2 quinoline), 13.13 (s, 1H, –CONH, D_2O exchangeable), 13.16 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 103.44, 109.03, 111.05, 111.90, 122.49, 125.25, 126.05, 128.22, 128.25, 137.91, 141.88, 142.23, 143.16, 145.64, 149.44, 151.29, 161.37; Anal. calcd. for $C_{19}H_{16}ClN_3O_4$ (385.80): C, 59.15; H, 4.18; N, 10.89. Found C, 59.34; H, 4.25; N, 10.89.

4.1.5.6. 7-Chloro-*N*'-(3,4,5-trimethoxybenzylidene)-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (**12f**).

White crystal (yield 82%), m.p. above 300 °C; IR (KBr, ν cm^{-1}): 3255 (NH, NH) and 1651, 1670 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 3.71 (s, 3H, OCH_3), 3.84 (s, 6H, OCH_3), 7.09 (s, 2H, H-2, H-6, 3,4,5-$(OCH_3)_3$-C_6H_2), 7.53- 7.55 (m, 1H, H-6 quinoline), 7.80 (s, 1H, H-8 quinoline), 8.27 (d, *J* = 8.8 Hz, 1H, H-5 quinoline), 8.35 (s, 1H, C<u>H</u>=N), 8.89 (s, 1H, H-2 quinoline), 12.91 (s, 1H, –

CONH, D_2O exchangeable), 13.16 (s, 1H, NH, D_2O exchangeable); Anal. calcd. for $C_{20}H_{18}ClN_3O_5$ (415.83): C, 57.77; H, 4.36; N, 10.11. Found C, 57.89; H, 4.39; N, 10.11.

4.1.5.7. 6-Chloro-*N*'-(4-chlorobenzylidene)-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (**12g**).

Yellow crystal (yield 81%), m.p. above 300 °C; IR (KBr, ν cm^{-1}): 3255 (NH, NH) and 1647, 1660 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 7.52 (d, 2H, *J* = 7.2 Hz, H-3, H-5, 4-Cl-C_6H_4), 7.77, (d, 2H, *J* = 7.2 Hz, H-2, H-6, 4-Cl-C_6H_4), 7.80-7.86 (m, 2H, H-7, H-8 quinoline), 8.20 (s, 1H, H-5 quinoline), 8.45 (s, 1H, C<u>H</u>=N), 8.90 (s, 1H, H-2 quinoline), 13.10 (s, 1H, –CONH, D_2O exchangeable), 13.16 (s, 1H, NH, D_2O exchangeable); Anal. calcd. for $C_{17}H_{11}Cl_2N_3O_2$ (360.18): C, 56.69; H, 3.08; N, 11.67. Found C, 56.69; H, 3.12; N, 11.67.

4.1.5.8. 6-Chloro-*N*'-(2,4-dichlorobenzylidene)-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (**12h**).

Yellow crystal (yield 82%), m.p. above 300 °C; IR (KBr, ν cm^{-1}): 3255 (NH, NH) and 1651, 1660 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 7.52 (d, 1H, *J* = 8.0 Hz, H-5, 2,4$(Cl)_2$-C_6H_3), 7.69 (s,1H, H-3, 2,4$(Cl)_2$-C_6H_3), 7.76-7.95 (m, 2H, H-7, H-8 quinoline), 8.00 (d, 1H, *J* = 8.4 Hz, H-6, 2,4$(Cl)_2$-C_6H_3), 8.22 (s, 1H, H-5 quinoline), 8.67 (s, 1H, C<u>H</u>=N), 8.81 (s, 1H, H-2 quinoline), 13.12 (s, 1H, –CONH, D_2O exchangeable), 13.28 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 110.60, 124.82, 127.20, 128.40, 128.78, 129.79, 130.54, 131.09, 133.59, 134.30, 135.56, 136.92, 138.21, 143.31, 145.26, 161.98, 163.35; Anal. calcd. for $C_{17}H_{10}Cl_3N_3O_2$ (394.64): C, 51.74; H, 2.55; N, 10.65. Found C, 51.97; H, 2.53; N, 10.68.

4.1.5.9. 6-Chloro-*N*'-(4-florobenzylidene)-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (**12i**).

Yellow crystal (yield 78%), m.p. above 300 °C; IR (KBr, ν cm^{-1}): 3317 (NH, NH) and 1651, 1670 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 7.26 (t, 2H, *J* = 8.8 Hz, H-2, H-6, 4-F-C_6H_4), 7.76 (dd, 2H, *J* = 8.8 Hz, H-3, H-5, 4-F-C_6H_4), 7.80- 7.84 (m, 2H, H-7, H-8 quinoline), 8.18 (s, 1H, *J* = 2.8 Hz, H-5 quinoline), 8.43 (s, 1H, C<u>H</u>=N), 8.87 (s, 1H, H-2 quinoline), 13.10 (s, 2H, NH, –CONH, D_2O exchangeable); Anal. calcd. for $C_{17}H_{11}ClFN_3O_2$ (343.74): C, 59.40; H, 3.23; N, 12.22. Found C, 59.74; H, 3.21; N, 12.22.

4.1.5.10. 6-Chloro-*N*'-(4-methylbenzylidene)-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (**12j**).

Yellow crystal (yield 86%), m.p. above 300 °C; IR (KBr, ν cm^{-1}): 3255 (NH, NH) and 1654, 1660 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.32 (s, 3H, CH_3) 7.23 (d, 2H, *J* = 8.0 Hz, H-3, H-5, 4- CH_3-C_6H_4), 7.61 (d, 2H, *J* = 8.0 Hz, H-2, H-6, 4-CH_3- C_6H_4), 7.74- 7.82 (m, 2H, H-7, H-8 quinoline), 8.17 (s, 1H, H-5 quinoline), 8.35 (s, 1H, C<u>H</u>=N), 8.86 (s, 1H, H-2 quinoline), 13.04 (s, 2H, NH, –

CONH, D_2O exchangeable); Anal. calcd. for $C_{18}H_{14}ClN_3O_2$ (339.78): C, 63.63; H, 4.15; N, 12.37. Found C, 63.90; H, 4.23; N, 12.37.

4.1.5.11. 6-Chloro-*N*'-(3,4-dimethoxybenzylidene)-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (**12k**).

Yellow crystal (yield 80%), m.p. above 300 °C; IR (KBr, ν cm^{-1}): 3255 (NH, NH) and 1658, 1670 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 3.79 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 7.01 (d, 1H, *J* = 8.4 Hz, H-5, 3,4-$(OCH_3)_2$-C_6H_3), 7.24 (d, 1H, *J* = 8.4 Hz, H-6, 3,4-$(OCH_3)_2$-C_6H_3), 7.35 (s, 1H, H-2, 3,4-$(OCH_3)_2$-C_6H_3), 7.76- 7.84 (m, 2H, H-7, H-8 quinoline), 8.33 (s, 1H, H-5 quinoline), 8.34 (s, 1H, C<u>H</u>=N), 8.86 (s, 1H, H-2 quinoline), 13.03 (s, 2H, NH, –CONH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 109.05, 110.97, 111.98, 122.42, 124.81, 126.87, 127.08, 127.49, 128.49, 130.36, 133.52, 138.34, 145.12, 148.50, 149.47, 151.21, 161.98; MS *m/z* %: 387.29 (M^++2, 7.78), 386.21 (M^+, 22.27), 81.08 (100%); Anal. calcd. for $C_{19}H_{16}ClN_3O_4$ (385.80): C, 59.15; H, 4.18; N, 10.89. Found C, 59.39; H, 4.12; N, 10.89.

4.1.5.12. 6-Chloro-*N*'-(3,4,5-trimethoxybenzylidene)-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (**12l**).

Yellow crystal (yield 85%), m.p. above 300 °C; IR (KBr, ν cm^{-1}): 3425 (NH, NH) and 1651, 1660 (C=O); ^{1}H NMR (DMSO-d_6) δ *ppm*: 3.71 (s, 3H, OCH_3), 3.84 (s, 6H, OCH_3), 7.09 (s, 2H, H-2, H-6, 3,4,5-$(OCH_3)_3$-C_6H_2), 7.75- 7.85 (m, 2H, H-7, H-8 quinoline), 8.20 (s, H-5 quinoline), 8.35 (s, 1H, C<u>H</u>=N), 8.89 (s, 1H, H-2 quinoline), 13.06 (s, 1H, –CONH, D_2O exchangeable), 13.13 (s, 1H, NH, D_2O exchangeable); MS *m/z* %: 417.14 (M^++2, 4.69), 415.12 (M^+, 9.56), 193.10 (100%); Anal. calcd. for $C_{20}H_{18}ClN_3O_5$ (415.83): C, 57.77; H, 4.36; N, 10.11. Found C, 57.98; H, 4.43; N, 10.37.

4.2 Biological evaluation

4.2.1. Antimicrobial activity

All strains were provided from culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Antibacterial and antifungal activities were expressed as the diameter of inhibition zones; agar well diffusion method was used. Holes (1 cm diameter) were digger in the agar using sterile cork borer in sterile malt agar plates for fungi and sterile nutrient agar plates for bacteria, which had previously been uniformly seeded with tested microorganisms. The holes were filled by fungal filtrates (100 µl). Plates were left in a cooled incubator at 4 °C for one hour for diffusion and then incubated at 37°C for tested bacteria and 28°C for tested fungi. Inhibition zones developed due to active antimicrobial metabolites were

measured after 24 hour of incubation for bacteria and 48 hour of incubation for fungi. Amphotericin B and ciprofloxacin were used as antifungal and antibacterial positive control; respectively. The experiment was performed in triplicate and the average zone of inhibition was calculated.

4.2.2. Minimum inhibitory concentration

MIC was performed by a serial dilution technique described by Irobi et al., [62], starting with 100 mmol concentration of all compounds dissolved in 1 mL DMSO and then reduced by successive twofold dilutions of stock solution using a calibrated micropipette. Amphotericin B and ciprofloxacin were used as the reference compounds for fungi and bacteria, respectively. The final solutions concentrations were 125, 62.50, 31.25, 15.63, 7.81, 3.90, 1.95, 0.98, 0.49, 0.24 and 0.12 µmol/mL. The microtiter plates were incubated at 37°C for tested bacteria and 28°C for tested fungi and were readied using microplate reader after 24 h for bacteria and after 48 h for fungi. In each case, triplicate tests were performed and the average was taken as final reading. MIC was expressed as the lowest concentration inhibiting test organism's growth [63].

4.2.3. Antimycobacterial activity

The *M. tuberculosis* (RCMB 010126) strain was provided from culture collection clinically isolated of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University (Cairo, Egypt). Isoniazide was used as reference drugs. Antimycobacterial activity of the synthesized compounds was evaluated using the microplate Alamar blue assay (MABA). Briefly, the inoculum was prepared from fresh LJ medium re-suspended in 7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC]), adjusted to a McFarland tube No. 1, and diluted 1:20; 100 µl was used as inoculum. Each compound and Isoniazide stock solution was thawed and diluted in 7H9-S at four-fold the final highest concentration tested. Serial two-fold dilutions of each compound and Isoniazide were prepared directly in a sterile 96-well microtiter plate using 100 µl 7H9-S. A growth control containing no antibiotic and a sterile control were also prepared on each plate. Sterile water was added to all perimeter wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags and incubated at 37^{o}C in normal atmosphere. After 7 days incubation, 30 ml of alamar blue solution was added to each well, and the plate was re-incubated overnight. A change in colour from blue (oxidised state) to pink (reduced) indicated the growth of bacteria, Percent inhibition was defined as: 1 − (mean of test well/mean of B wells) × 100. the MIC was defined as the lowest concentration of drug that prevented this change in colour [64].

Mammalian cell lines: WI-38 cells (human lung fibroblast normal cell line), were obtained from VACSERA Tissue Culture Unit.

Chemicals Used: Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA).

Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza.

Crystal violet stain (1%): It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with ddH2O and filtered through a Whatmann No.1 filter paper.

Cell line Propagation:

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin. All cells were maintained at 37ºC in a humidified atmosphere with 5% CO2 and were subcultured two times a week.

Cytotoxicity evaluation using viability assay: For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×104 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37ºC in a humidified incubator with 5% CO2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. After incubation of the cells for at 37°C, various concentrations of sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method.

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated. The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [1-(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC50), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA) [65,66].

All title compounds were Sketched in 2D and minimized by the protocol “Prepared Ligands” in the DS 4.0 software (Discovery Studio 4.0, Accelrys, Co. Ltd). The antifungal, antibacterial and antimycobacterial activities of the compounds MIC were converted into the logarithmic scale PMIC (PMIC= -log MIC) and then proceed to the QSAR analysis as the response variables. The data set was divided into the two subsets (Training and test set). Training set for antifungal and antibacterial was 17 compounds and test set were 3 compounds. While for antimycobacterial it was 20 compounds and test set, 4 compounds. The test set compounds were selected manually considering the distribution of biological data and structural diversity. The training set was used to build a regression model, and the test set was used to evaluate the predictive ability of the model obtained [67].

In this study, sixty eight molecular descriptors representing thermodynamic, electronic, spatial, structural, thermodynamic, geometric, topological and quantum mechanical properties were calculated using “Calculate Molecular Properties” protocol of the Discovery Studio 4.0. where all the descriptor values for the molecules were considered as independent variables while, the inhibitory concentration results (pMIC) were taken as dependent variables.

Then, by applying the “Genetic Function Approximation” module, certain descriptors were selected then used to build a significant QSAR model by one of the best methods “Multiple Linear Regression” (MLR) [68].

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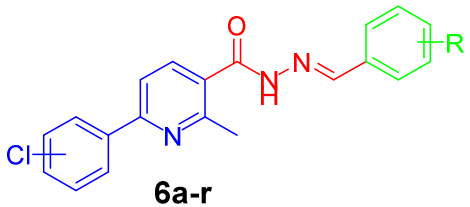
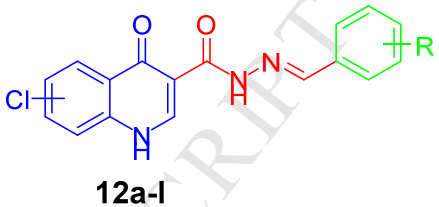
Table 1. Antimicrobial activity of the synthesized compounds (**6a-r**) and (**12a-l**) against the pathological organisms expressed as inhibition diameter zones in millimeters (mm) based on well diffusion assay.



6a-r



12a-l

Comp.	Cl	R	Fungi		Gram Positive Bacteria		Gram negative Bacteria	
			Af	**Ca**	**Sp**	**Sa**	**Pa**	**Ec**
6a	3-Cl	4-Cl	20.3 ± 0.25	17.9 ± 0.44	21.3 ± 0.68	19.6 ± 0.36	NA	23.7 ± 0.58
6b	3-Cl	2,4-$(Cl)_2$	NA	NA	19.3 ± 0.58	11.4 ± 0.44	NA	NA
6c	3-Cl	4-F	NA	NA	NA	NA	NA	NA
6d	3-Cl	4-CH_3	NA	NA	13.6 ± 0.36	12.6 ± 0.19	NA	NA
6e	3-Cl	3,4-$(OCH3)_2$	19.3 ± 0.58	17.2 ± 1.50	19.6 ± 0.44	17.9 ± 0.28	NA	21.6 ± 0.44
6f	3-Cl	3,4,5-$(OCH3)_3$	23.3 ± 0.58	21.4 ± 1.20	23.1 ± 1.50	21.3 ± 1.20	NA	25.3 ± 0.58
6g	4-Cl	4-Cl	NA	NA	NA	NA	NA	NA
6h	4-Cl	2,4-$(Cl)_2$	19.3 ± 0.53	17.4 ± 0.44	21.3 ± 0.53	19.3 ± 0.44	NA	21.3 ± 0.23
6i	4-Cl	4-F	NA	NA	NA	NA	NA	NA
6j	4-Cl	4-CH_3	NA	NA	NA	NA	NA	NA
6k	4-Cl	3,4-$(OCH3)_2$	NA	NA	NA	NA	NA	NA
6l	4-Cl	3,4,5-$(OCH3)_3$	21.8 ± 0.32	20.4 ± 1.50	21.3 ± 0.19	23.4 ± 0.37	NA	21.3 ± 0.24
6m	4-Cl	4-OH	19.3 ± 0.63	17.3 ± 1.50	20.3 ± 0.72	18.3 ± 1.50	NA	19.2 ± 0.58
6n	4-Cl	4-OCH_3	20.9 ± 0.58	19.2 ± 0.63	21.3 ± 1.20	20.1 ± 0.72	NA	20.9 ± 0.58
6o	4-Cl	2-OH-3-OCH_3	21.3 ± 0.63	20.3 ±0.72	22.3 ± 1.50	20.5 ± 0.63	NA	21.5 ± 0.58
6p	4-Cl	2,5-$(OCH3)_2$	24.3 ± 1.20	22.5 ± 0.58	24.3 ± 0.72	22.3 ± 1.50	NA	24.3 ± 0.58
6q	3-Cl	4-OCH_3	20.6 ± 0.63	18.2 ± 0.58	20.9 ± 1.50	19.6 ± 1.20	NA	20.3 ± 0.63
6r	3-Cl	2,5-$(OCH3)_2$	22.6 ± 0.58	21.2 ± 0.36	22.5 ± 0.63	21.3 ± 0.72	NA	22.3 ± 1.20
AB			23.7 ± 1.20	25.4 ± 0.58				
AMP					23.8 ± 1.20	27.4 ± 0.72		
CF							20.6± 1.20	23.4 ± 0.63

Continue Table 1. Antimicrobial activity of the synthesized compounds against the pathological organisms expressed as inhibition diameter zones in millimeters (mm) based on well diffusion assay.

Comp.	Cl	R	Fungi		Gram Positive Bacteria		Gram negative Bacteria	
			Af	**Ca**	**Sp**	**Sa**	**Pa**	**Ec**
12a	7-Cl	4-Cl	17.3 ± 0.58	15.2 ± 1.20	17.1 ± 0.44	16.2 ± 0.63	NA	18.3 ± 1.50
12b	7-Cl	2,4-$(Cl)_2$	20.3 ± 0.36	19.6 ± 0.35	22.1 ± 0.55	20.6 ± 0.52	NA	24.3 ± 0.58
12c	7-Cl	4-F	16.8 ± 0.63	14.4 ± 0.58	16.9 ± 1.50	16.2 ± 1.20	NA	12.5 ± 0.63
12d	7-Cl	4-CH_3	13.7 ± 0.36	14.5 ± 0.25	13.9 ± 0.29	14.4 ± 0.38	NA	15.0 ± 0.25
12e	7-Cl	3,4-$(OCH3)_2$	20.3 ± 0.58	16.2 ± 1.20	17.3 ± 0.63	20.6 ± 0.58	NA	20.3 ± 1.20
12f	7-Cl	3,4,5-$(OCH3)_3$	NA	NA	NA	NA	NA	NA
12g	6-Cl	4-Cl	16.3 ± 0.19	13.6 ± 1.20	17.3 ± 0.63	15.3 ± 1.50	NA	20.6 ± 0.58
12h	6-Cl	2,4-$(Cl)_2$	16.9 ± 1.20	14.1 ± 1.50	17.5 ± 0.63	15.7 ± 0.58	NA	20.7 ± 1.20
12i	6-Cl	4-F	11.3 ± 1.20	10.3 ± 0.58	12.4 ± 1.50	10.6 ± 0.63	NA	12.7 ± 1.50
12j	6-Cl	4-CH_3	10.3 ± 0.63	12.1 ± 0.43	20.4 ± 0.35	NA	NA	NA
12k	6-Cl	3,4-$(OCH3)_2$	18.4 ± 0.58	16.9 ± 0.63	19.1 ± 1.20	17.2 ± 1.50	NA	21.3 ± 1.20
12l	6-Cl	3,4,5-$(OCH3)_3$	18.1 ± 0.63	16.3 ± 0.36	18.4 ± 0.67	17.3 ± 0.56	NA	19.3 ± 0.72
AB			23.7 ± 1.20	25.4 ± 0.58				
AMP					23.8 ± 1.20	27.4 ± 0.72		
CF							20.6± 1.20	23.4 ± 0.63

NA: No Activity.

The screening organisms, Mould: *Aspergillus fumigatus* (RCMB 02568, An), An Yeasts: *Candida albicans* (RCMB 05036, Ca), Gram-positive bacteria: *Streptococus pneumoniae* (RCMB 010010, Sp), and *Staphylococcus aureus* (RCMB 010028, Sa). Gram-negative bacteria: *Pseudomonas aeruginosa* (RCMB 010043, Pa), and *Escherichia coli* (RCMB 010052, Ec), **AB: Amphotericin B, AMP: Ampicillin, CF: Ciprofloxacin**

Table 2. Antimicrobial activity as MICs (µg/mL) of tested standards and best active compounds against tested microorganisms

Comp.	Fungi		Gram Positive Bacteria		Gram negative Bacteria	
	Af	**Ca**	**Sp**	**Sa**	**Pa**	**Ec**
6a	1.95	3.9	1.95	1.95	NA	**0.49**
6e	3.9	15.63	3.9	7.81	NA	1.95
6f	**0.98**	**0.49**	**0.49**	1.95	NA	**0.49**
6h	3.9	15.63	1.95	3.9	NA	1.95
6l	**0.98**	1.95	1.95	**0.98**	NA	1.95
6m	3.9	15.63	3.9	7.81	NA	3.9
6n	1.95	3.9	1.95	3.9	NA	1.95
6o	1.95	3.9	0.98	1.95	NA	1.95
6p	**0.98**	**0.98**	**0.49**	**0.98**	NA	**0.49**
6q	1.95	7.81	3.9	3.9	NA	3.9
6r	**0.98**	1.95	**0.98**	1.95	NA	**0.98**
12a	15.63	31.25	15.63	31.25	NA	7.81
12b	3.9	3.9	**0.98**	1.95	NA	**0.49**
12d	62.5	62.5	62.5	62.5	NA	62.5
12e	3.9	31.25	15.63	1.95	NA	3.9
12g	31.25	62.5	15.63	31.25	NA	1.95
12h	15.63	62.5	7.81	15.63	NA	1.95
12i	15.63	31.25	15.63	31.25	NA	62.5
12k	7.81	15.63	3.9	15.63	NA	1.95
12l	7.81	31.25	7.81	15.63	NA	3.9
AB	1.95	0.98				
AMP			0.98	0.98		
CF					1.95	0.98

NA: No Activity.

The screening organisms, Mould: *Aspergillus fumigatus* (RCMB 02568, Af), An Yeasts: *Candida albicans* (RCMB 05036, Ca), Gram-positive bacteria: *Streptococus pneumoniae* (RCMB 010010, Sp), and *Staphylococcus aureus* (RCMB 010028, Sa). Gram-negative bacteria: *Pseudomonas aeruginosa* (RCMB 010043, Pa), and *Escherichia coli* (RCMB 010052, Ec), **AB: Amphotericin B, AMP: Ampicillin, CF: Ciprofloxacin**

Table 3. Anti-tubercular activities of all the synthesized compounds.

Comp.	I.Z	MIC
6a	43.25 ± 0.58	6.24
6b	41.98 ± 0.63	6.24
6c	52.34 ± 1.20	3.12
6d	61.24 ± 1.50	1.56
6e	73.58 ± 0.72	**0.78**
6f	70.48 ± 0.63	**0.78**
6g	36.24 ± 1.50	12.48
6h	35.38 ± 1.50	12.48
6i	40.31 ± 0.58	6.24
6j	26.35 ± 1.50	49.92
6k	61.32 ± 0.72	1.56
6l	68.42 ± 1.20	**0.78**
6m	53.21 ± 1.20	3.12
6n	70.34 ± 0.72	**0.78**
6o	80.63 ± 0.63	**0.39**
6p	85.32 ± 1.50	**0.39**
6q	60.42 ± 0.58	1.56
6r	82.35 ± 1.20	**0.39**
12a	19.35 ± 1.50	99.84
12b	NA	NA
12c	36.42 ± 1.50	12.48
12d	NA	NA
12e	NA	NA
12f	42.65 ± 1.20	6.24
12g	17.80 ± 0.58	99.84
12h	18.30 ± 0.63	99.84
12i	NA	NA
12j	NA	NA
12k	31.25 ± 1.20	24.96
12l	NA	NA
Isoniazide	NA	**0.75**

Table 4. In vitro cytotoxic activities of compounds **6d, 6e, 6f, 6k, 6l, 6m, 6n, 6o, 6p ,6q** and **6r** .

Comp.	MIC	IC50	Selectivity index
6d	1.56	**500**	**320.51**
6e	**0.78**	**168**	**215.38**
6f	**0.78**	**495**	**634.61**
6k	1.56	**443**	**283.97**
6l	**0.78**	**41.1**	**52.69**
6m	3.12	**500**	**160.25**
6n	**0.78**	**500**	**641**
6o	**0.39**	**500**	**1282.05**
6p	**0.39**	**500**	**1282.05**
6q	1.56	**500**	**320.51**
6r	**0.39**	**188**	**482.05**

Table 5. Experimental activities of the synthesized derivatives against the predicted activity according to equations 1, 2 and 3.

Comp.	*Aspergillus fumigatus*			*Streptococus pneumoniae*			*Mycobacterium tuberculosis*		
	Experimental Activity (pMIC)	Predicted Activity (pMIC)	Residual	Experimental Activity (pMIC)	Predicted Activity (pMIC)	Residual	Experimental Activity (pMIC)	Predicted Activity (pMIC)	Residual
6a	-0.2900	-0.4888	0.1987	-0.2900	-0.3574	0.0673	-0.7952	-0.9183	0.1231
6b	–	–	–	–	–	–	-0.7952	-1.0454	0.2502
6c	–	–	–	–	–	–	-0.4942	-0.5913	0.0972
6d	–	–	–	–	–	–	-0.1931	-0.1613	-0.0318
6e	-0.5911	-0.3300	-0.2610	-0.5911	-0.5282	-0.0629	0.1079	0.0911	0.0168
6f	0.0088	–	–	0.3098	–	–	0.1079	–	–
6g	–	–	–	–	–	–	-1.0962	-0.8628	-0.2334
6h	-0.5911	-0.3059	-0.2852	-0.2900	-0.0159	-0.2742	-1.0962	-1.0541	-0.0421
6i	–	–	–	–	–	–	-0.7952	-0.5175	-0.2777
6j	–	–	–	–	–	–	-1.6983	–	–
6k	–	–	–	–	–	–	-0.1931	0.1793	-0.3725
6l	0.0088	-0.0393	0.0480	-0.2900	-0.2315	-0.0585	0.1079	0.3108	-0.2029
6m	-0.5911	-0.4731	-0.1179	-0.5911	-0.3835	-0.2076	-0.4942	-0.3083	-0.1859
6n	-0.29	-0.2352	-0.0549	-0.2900	-0.0982	-0.1919	0.1079	-0.0286	0.1365
6o	-0.29	-0.266	-0.0241	0.0088	-0.0682	0.0769	0.4089	0.2532	0.1557
6p	0.0088	-0.2833	0.292	0.3098	-0.2732	0.5830	0.4089	0.0946	0.3143
6q	-0.29	-0.5598	0.2698	-0.5911	-0.7143	0.1232	-0.1931	-0.2132	0.0201
6r	0.0088	0.0242	-0.0155	0.0088	0.1442	-0.1354	0.4089	0.1811	0.2278
12a	-1.194	-1.3242	0.1302	-1.1940	-1.3789	0.185	-1.9993	-1.7504	-0.2489
12b	-0.5911	-0.8347	0.2436	0.0088	-0.4727	0.4814	–	–	–
12c	–	–	–	–	–	–	-1.1086	-1.5191	0.4106
12d	-1.7959	-1.3921	-0.4038	-1.7959	-1.4393	-0.3565	–	–	–
12e	-0.5911	-0.8823	0.2912	-1.1940	-1.0416	-0.1524	–	–	–

12f	–	–	–	–	–	–	-0.8075	-0.9475	0.1400
12g	-1.4949	–	–	-1.194	–	–	-1.9993	–	–
12h	-1.194	–	–	-0.8927	–	–	-1.9993	–	–
12i	-1.194	-1.1733	-0.0206	-1.194	-1.0582	-0.1357	–	–	–
12k	-0.8927	-0.9549	0.0622	-0.8927	-1.1794	0.2867	-1.3972	-1.1001	-0.2971
12l	-0.8927	-0.5398	-0.3529	-0.8927	-0.6642	-0.2285	–	–	–

Table 6. External validation for the established QSAR model.

Comp.	***Aspergillus fumigatus***			***Streptococus pneumoniae***			***Mycobacterium tuberculosis***		
	Experimental Activity (pMIC)	Predicted Activity (pMIC)	Residual	Experimental Activity (pMIC)	Predicted Activity (pMIC)	Residual	Experimental Activity (pMIC)	Predicted Activity (pMIC)	Residual
6f	0.0088	-0.0801	0.0888	0.3098	-0.1066	0.4164	0.1079	-0.6399	0.7478
6j	----------	----------	----------	----------	----------	----------	-1.6983	-0.8361	-0.8622
12g	-1.4949	-1.1306	-0.3643	-1.194	-0.5137	-0.6802	-1.9993	-1.9967	-0.0026
12h	-1.194	-1.4694	0.2754	-0.8927	-1.1564	0.2638	-1.9993	-2.1163	0.1170

Table 7. Computer aided ADME study of the active derivatives.

Compound	Alog*P*98[a]	PSA_2D[b]	Solubility[c]	Solubility level[d]	Absorption level[e]	CYP2D6[f]	CYP2D6 probability[g]
6a	5.091	52.695	-6.066	1	0	0	0.198
6b	5.755	52.695	-6.761	1	0	0	0.227
6c	4.632	52.695	-5.665	2	0	0	0.306
6d	4.912	52.695	-5.826	2	0	0	0.277
6e	4.393	70.555	-5.317	2	0	0	0.188
6f	4.377	79.485	-5.282	2	0	0	0.356
6g	5.091	52.695	-6.056	1	0	0	0.277
6h	5.755	52.695	-6.752	1	0	0	0.366
6i	4.632	52.695	-5.656	2	0	0	0.366
6j	4.912	52.695	-5.816	2	0	0	0.277
6k	4.393	70.555	-5.306	2	0	0	0.277
6l	4.377	79.485	-5.27	2	0	0	0.336
6m	4.184	73.51	-4.949	2	0	0	0.366
6n	4.41	61.625	-5.332	2	0	0	0.366
6o	4.168	82.44	-4.995	2	0	0	0.366
6p	4.168	82.44	-5.001	2	0	0	0.366
6q	4.41	61.625	-5.342	2	0	0	0.227
6r	4.393	70.555	-5.35	2	0	0	0.287
12a	3.355	71.545	-4.891	2	0	0	0.079
12b	4.019	71.545	-5.695	2	0	0	0.198
12c	2.896	71.545	-4.49	2	0	0	0.168
12d	3.177	71.545	-4.566	2	0	0	0.168
12e	2.658	89.405	-4.279	2	0	0	0.346
12f	2.642	98.335	-4.363	2	0	0	0.366
12g	3.355	71.545	-4.897	2	0	0	0.108
12h	4.019	71.545	-5.702	2	0	0	0.227
12i	2.896	71.545	-4.496	2	0	0	0.198
12j	3.177	71.545	-4.572	2	0	0	0.178
12k	2.658	89.405	-4.287	2	0	0	0.346

12l	2.642	98.335	-4.372	2	0	0	0.336

[a] Lipophilicity descriptor.
[b] Polar surface area.
[c] Solubility parameter.(0 : - 2 = optimal, -2 : -4 = good, -4 : -6 = low, -6 : -8 = very low)
[d] Solubility level. (0 = extremely low, 1 = very low but possible, 2 = low, 3 = good, 4 = optimal).
[e] Absorption level. (0 = good, 1 = moderate, 2 = low, 3 = very low)

[f] CYP2D inhibition. (0 = non inhibitor, 1 = inhibitor)

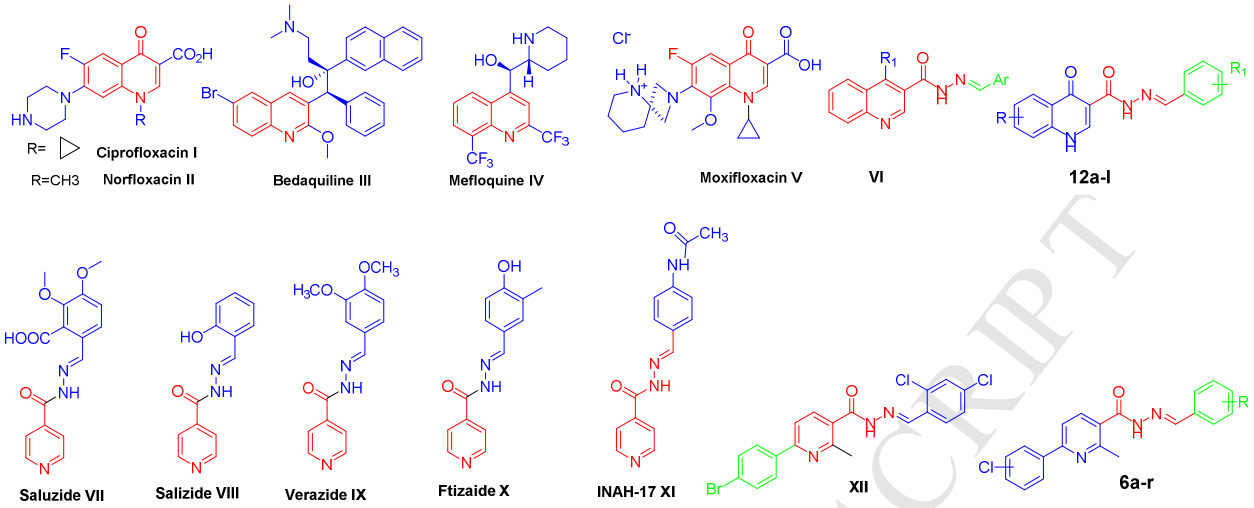


Figure 1. Structures of some representative antitubercular and antibacterial drugs **I–XII** and the target derivatives **6a–r** and **12a–l**.

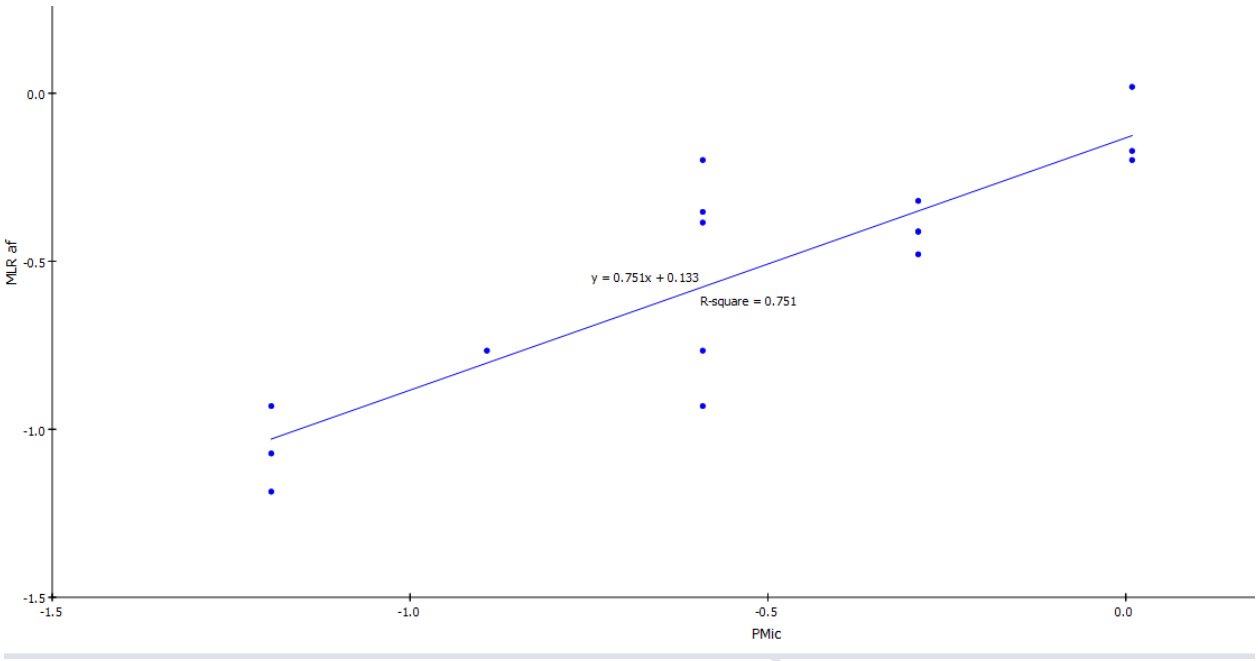


Figure 2. Predicted versus experimental pMIC of the tested compounds against ***Aspergillus fumigatus*** according to Equation **1**. (r^2 = 0.751)

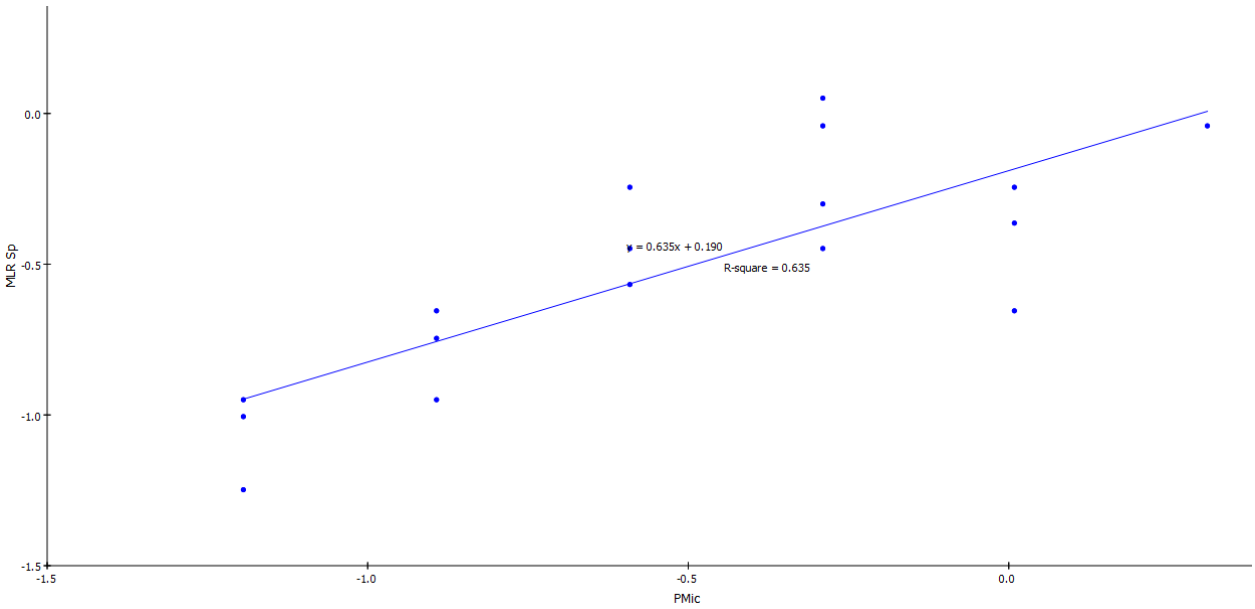


Figure 3. Predicted versus experimental pMIC of the tested compounds against ***Streptococus pneumoniae*** according to Equation **2**. (r^2 = 0.635)

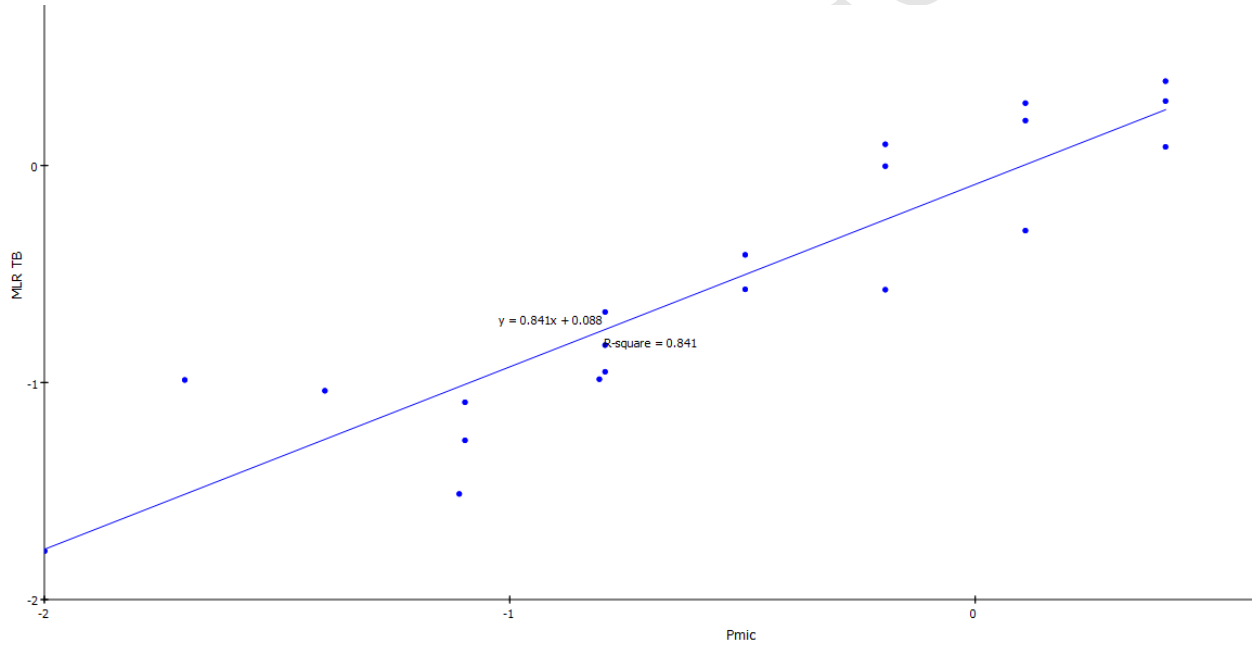


Figure 4. Predicted versus experimental pMIC of the tested compounds against ***M. tuberculosis*** according to Equation **3**. (r^2 = 0.841)

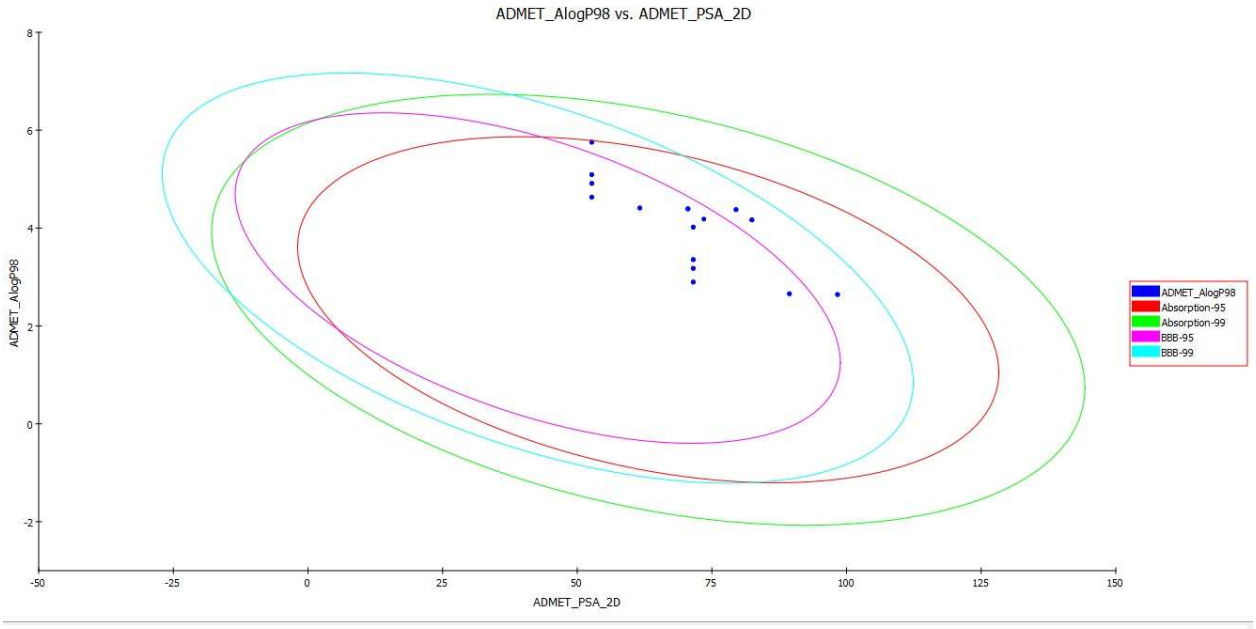
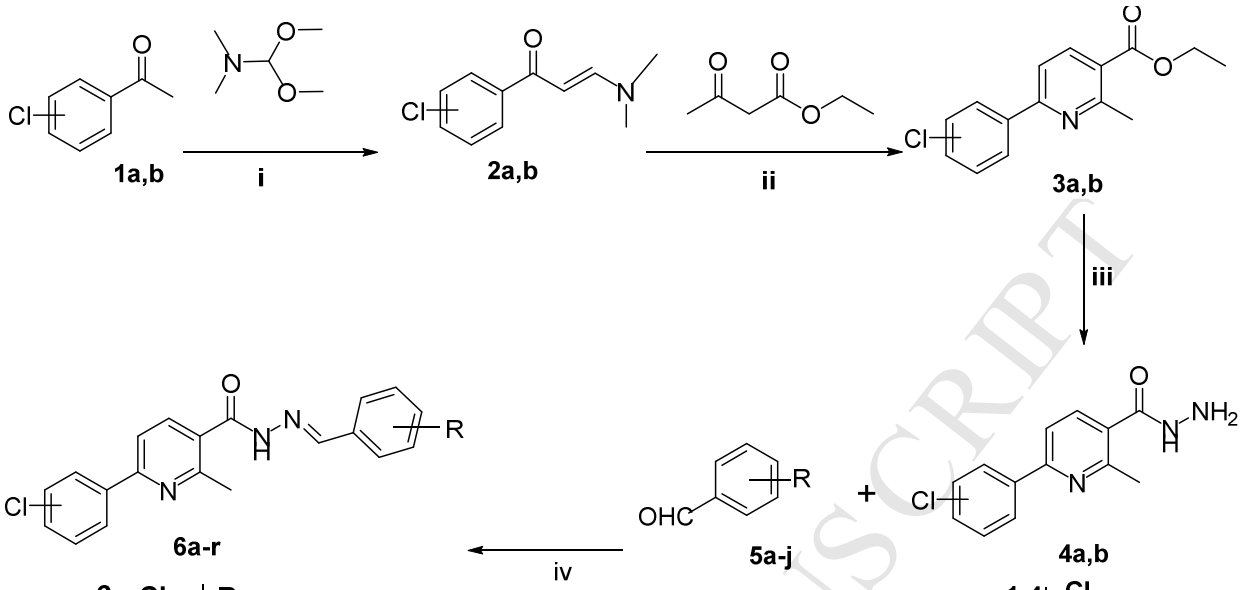


Figure 5: The results of ADMET Studies.

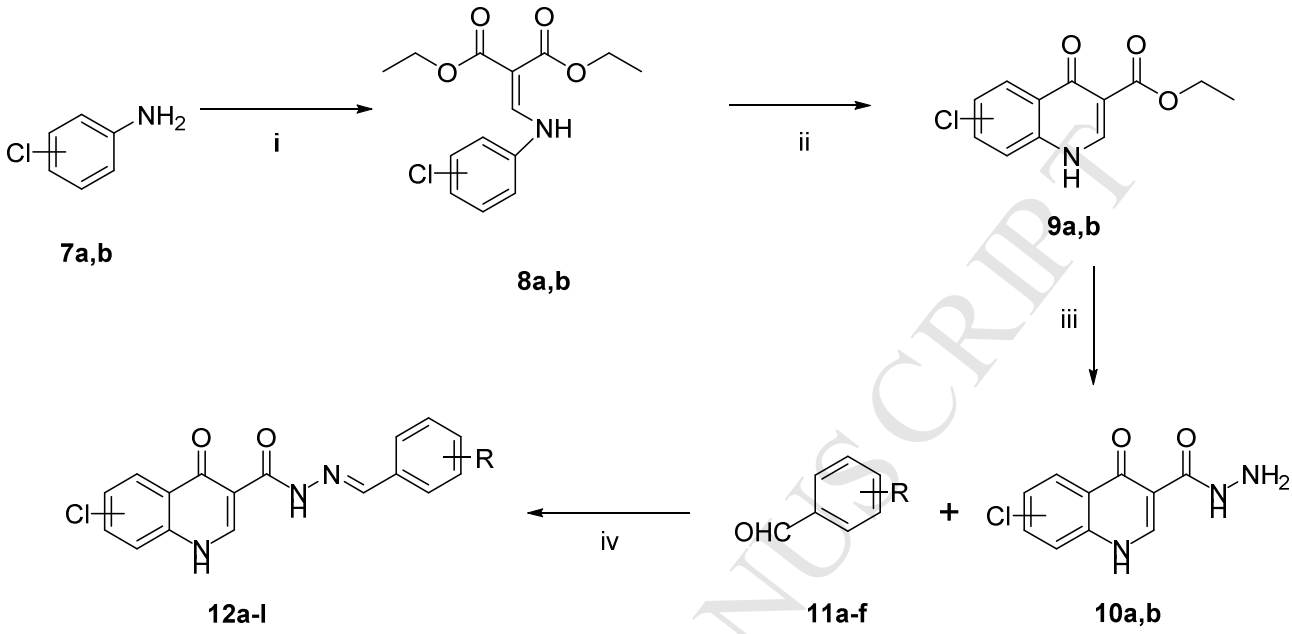


6	Cl	R
a	3-Cl	4-Cl
b	3-Cl	2,4-$(Cl)_2$
c	3-Cl	4-F
d	3-Cl	4-CH_3
e	3-Cl	3,4-$(OCH_3)_2$
f	3-Cl	3,4,5-$(OCH_3)_3$
g	4-Cl	4-Cl
h	4-Cl	2,4-$(Cl)_2$
i	4-Cl	4-F
j	4-Cl	4-CH_3
k	4-Cl	3,4-$(OCH_3)_2$
l	4-Cl	3,4,5-$(OCH_3)_3$
m	4-Cl	4-OH
n	4-Cl	4-OCH_3
o	4-Cl	2-OH-3-OCH_3
p	4-Cl	2,5-$(OCH_3)_2$
q	3-Cl	4-OCH_3
r	3-Cl	2,5-$(OCH_3)_2$

1-4	Cl
a	*3-Cl*
b	*4-Cl*

Scheme 1. Synthesis of nicotinic acid hydrazones **6a-r**.

Reagents and conditions: i: DMF/DMA, reflux 8h, (70- 77%); ii: NH_4OAc/ AcOH/ reflux 5h, (80-83%); iii: $NH_2NH_2.H_2O$/ reflux 3 h, (85-90 %); vi: EtOH/AcOH (catalytic) / reflux 4 h, (60- 90%).



7a,b **8a,b** **9a,b** **12a-l** **11a-f** **10a,b**

12	Cl	R
a	7-Cl	4-Cl
b	7-Cl	2,4-$(Cl)_2$
c	7-Cl	4-F
d	7-Cl	4-CH_3
e	7-Cl	3,4-$(OCH_3)_2$
f	7-Cl	3,4,5-$(OCH_3)_3$
g	6-Cl	4-Cl
h	6-Cl	2,4-$(Cl)_2$
i	6-Cl	4-F
j	6-Cl	4-CH_3
k	6-Cl	3,4-$(OCH_3)_2$
l	6-Cl	3,4,5-$(OCH_3)_3$

8-10	Cl
a	7-Cl
b	6-Cl

Scheme 2. Synthesis of 1,4-dihydro-4-oxo-quinoline-3-carbohydrazide derivatives **12a-l**.

Reagents and conditions: i: Diethyl ethoxy methylene malonate, reflux 3h, (70- 77%); ii: Diphenyl ether reflux 2 h, (75- 80%); iii: Hydrazine hydrate reflux 3 h, (80- 83%); iv: DMF reflux 2 h, (78- 86%).

- This work deals with a novel series of pyridine and quinolone derivatives and their evaluation as antimicrobial and antimycobacterial agents.
- A SAR was discussed and a QSAR study was carried out to correlate this activity.
- All the requirements of the reviewers were carried out, underlined and a new table added concerning the cytotoxic study.