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Synthesis, anti-tuberculosis activity, and 3D-QSAR study of ring-substituted-2/4-quinolinecarbaldehyde derivatives

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Abstract—We have previously identified ring-substituted quinolines as a new structural class of anti-tuberculosis agents. In our ongoing efforts at structural optimization of this class, four series of ring-substituted-2/4-quinolinecarbaldehyde derivatives were synthesized. All twenty-four compounds were synthesized using short and convenient one to two high yielding steps. The newly synthesized compounds were tested in vitro against drug-sensitive *Mycobacterium tuberculosis H37Hv* strain. Several derivatives were found to be promising inhibitors of *M. tuberculosis*. For example, derivatives **4a**–**c** (Series 2), **7a**–**d** (Series 3), and **8a**–**b** (Series 4) displayed >90% inhibition at 6.25 μg/mL in the primary assay. The most active compounds, *N*-(2-fluorophenyl)-*N*′-quinolin-2-ylmethylene-hydrazine (**4a**), *N*-(2-adamantan-1-yl-quinolin-4-ylmethylene)-*N*′-(4-fluorophenyl)hydrazine (**7c**), and *N*-(2-cyclohexyl-quinolin-4-ylmethylene)-*N*′-(2-fluorophenyl)hydrazine (**8a**), exhibited 99% inhibition at the lowest tested concentration of 3.125 μg/mL against drug-sensitive *M. tuberculosis H37Rv* strain. The similarity index based on steric and electrostatic features of the molecules was used, in conjunction with principal component analysis and linear discriminant analysis, successively to classify the molecules based on their activity into two classes. This classification method gives us confidence in predicting the activity class of any new unsynthesized molecule belonging to these series.

1. Introduction

Tuberculosis (TB) remains a health problem of enormous dimension throughout the world. It has been estimated that every year 8 million people develop active TB and nearly 25% of them succumb to the disease—accounting for approximately 5500 deaths every day.[1,2] The situation has been further aggravated by the association of acquired immunodeficiency syndrome (AIDS) with TB.[3] Since the containment of the TB infection in an individual depends on intact cellular immunity, human immunodeficiency virus (HIV), due to its ability to destroy the immune system, has now emerged as the most significant risk factor for progression of dormant TB to clinical infection. HIV-associated TB and the emergence of multi-drug resistant TB (MDR-TB) increase both the magnitude and severity of the disease. Although drug resistance in *Mycobacterium tuberculosis* surfaced shortly after the introduction of streptomycin in the late 1940s, the current threat is the emergence of strains resistant to the two most potent anti-TB drugs, isoniazid and rifampicin. Even though TB is 100% curable using first-line drugs, drug resistance easily develops due to various reasons including poor adherence to treatment, lack of adequate supervision, interruption of chemotherapy due to side effects, and the use of anti-TB drugs for indications other than TB. The response of patients with MDR-TB to treatment with expensive and toxic second-line drugs is poor and the mortality rate is about 50%.

Recent advances such as the availability of the TB genome sequence have provided a wide range of novel targets for drug design,[4] yet, no new tuberculosis-specific drug has reached the clinic in the past forty years.[5] This underscores an urgent and pressing need to discover new structural classes of anti-tuberculosis agents which may replace and/or supplement the current drug regimens.

Keywords: Tuberculosis; Synthesis; Ring-substituted-2/4-quinolinecarbaldehyde derivatives; 3D-QSAR study.
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We have previously reported ring-substituted quinolines as a promising new structural class of anti-tuberculosis agents, which emerged from a broad structure-directed screening approach of new chemical entities against various pathogens including *M. tuberculosis*.[6–9] Most of the ring-substituted quinolines reported by us were synthesized in 1–3 overall steps, and thus are attractive lead compounds for the additional structural optimization. Our efforts to optimize the structure and biological activity have resulted in several potential compounds. In particular, two analogs, 2,8-dicyclopentyl-4-methylquinoline (DCMQ),[6] and 4-(1-adamantyl)-2-quinolinecarbohydrazide (AQCH)[9] (Fig. 1), displayed most promising activities against both drug-sensitive and drug-resistant *M. tuberculosis H37Rv* strains.

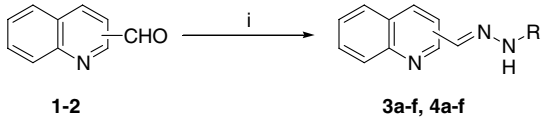
More recently, in continuation of our anti-tuberculosis drug discovery program, we observed moderate inhibitory activity with 4-quinolinecarbaldehyde (**1**, 61% inhibition at 6.25 μg/mL) and 2-quinolinecarbaldehyde (**2**, 30% inhibition at 6.25 μg/mL) against drug-sensitive *M. tuberculosis H37Rv* strain. This observation prompted us to initiate additional structural optimization of **1** and **2**. We derivatized carbaldehyde group of **1** and **2** and subsequently exploited homolytic free radical reaction[10,11] for the direct and regiospecific incorporation of various cycloalkyl groups in the quinoline ring to produce ring-substituted-2/4-quinolinecarbaldehydes. The information regarding potential drug-target for the ring-substituted quinoline class of anti-tuberculosis agents mainly remains unknown. In such a case, we applied indirect ligand-based 3D-QSAR analysis approach to assist us in understanding the SAR of the synthesized molecules. This approach can also serve as a guide in the design of more potent inhibitors.

In this work, we report synthesis, biological activity, and 3D-QSAR analysis of four new series (Series 1–4) of ring-substituted-2/4-quinolinecarbaldehyde derivatives (Fig. 2). It is generally observed that presence of an electronegative fluorine in a molecule can profoundly affect its pK_a, chemical reactivity, and biological activity. Keeping this observation in mind, synthetic derivatization of the ring-substituted-2/4-carbaldehydes with the 2/3/4-fluorophenylhydrazines and phenylhydrazine was studied to determine the effect of fluoro group on anti-TB activity. At the same time, synthetic derivatization of the carbaldehyde group was also achieved with the aliphatic electron-withdrawing commercially available formic hydrazide and acetylhydrazide.

2. Chemistry

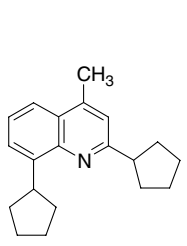
Commercially available 4-quinolinecarbaldehyde (**1**) and 2-quinolinecarbaldehyde (**2**) upon reaction with various commercially available aromatic and aliphatic hydrazines in the presence of abs ethyl alcohol at 80 °C for 8 h afforded *N*-(alkyl)-*N*′-quinolin-4-ylmethylene-hydrazines/hydrazides **3a–f** and *N*-(alkyl)-*N*′-quinolin-2-ylmethylene-hydrazines/hydrazides **4a–f**, respectively (Scheme 1).

We have earlier reported homolytic free radical reaction of various ring-functionalized quinolines which involves heating a mixture of the requisite quinoline derivative and suitable alkylcarboxylic acid, in the presence of the $AgNO_3$ and ammonium persulfate in H_2SO_4 and CH_3CN as solvent at 70–80 °C for 15 min.[9] However, the application of similar conditions for the ring-alkylation of **1** and **2** did not produce the desired products,

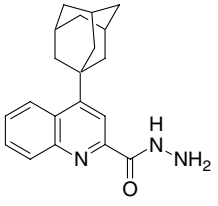


1-2 **3a-f, 4a-f**

Scheme 1. Reagents and conditions: (i) NH_2NHR, abs EtOH, 80 °C, 8 h.

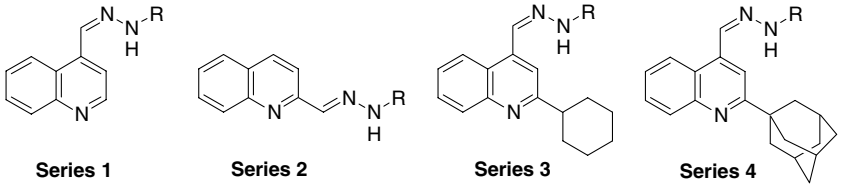


2,8-Dicyclopentyl-4-methylquinoline (DCMQ)



4-(1-Adamantyl)-2-quinolinecarbohydrazide (AQCH)

Figure 1. Structures of promising anti-TB ring-substituted quinolines.



Series 1 **Series 2** **Series 3** **Series 4**

Figure 2. General structures of synthesized ring substituted quinolines.

presumably due to the instability of the carbaldehyde group under the oxidative nature of the reaction conditions and high temperature. We then varied the reaction conditions and observed that best results were observed by carrying out the reaction at ambient temperature for 30 min. Thus, 2-alkyl-4-quinolinecarbaldehydes **5** and **6** were conveniently synthesized from **1** by homolytic free radical reaction using requisite alkylcarboxylic acid in the presence of a catalytic amount of $AgNO_3$ and ammonium persulfate in H_2SO_4 and CH_3CN as solvent at ambient temperature for 30 min (Scheme 2).

Unfortunately, the modified reaction conditions applicable for the direct ring-substitution of 4-quinolinecarbaldehyde (**1**) could not be extended to 2-quinolinecarbaldehyde (**2**). In the latter case, all attempts lead to the formation of unidentifiable high molecular weight products possibly due to polymerization, along with some quantity of unreacted starting material and was not pursued further. Finally, 2-alkyl-quinoline-4-carbaldehydes **5** and **6** upon reaction with various commercially available aliphatic/aromatic hydrazines in the presence of abs ethyl alcohol at 80 °C for 8 h produced *N*-(2-adamantan-1-yl-quinolin-4-yl-methylene)-N′-substituted hydrazines/hydrazides **7a**–**f** and *N*-(2-cyclohexyl-quinolin-4-yl-methylene)-N′-substituted hydrazines/hydrazides **8a**–**f** (Scheme 1).

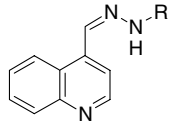
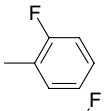
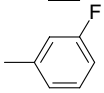
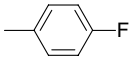
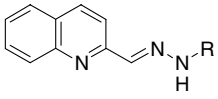
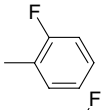
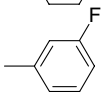
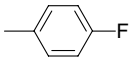
3. Biological activity

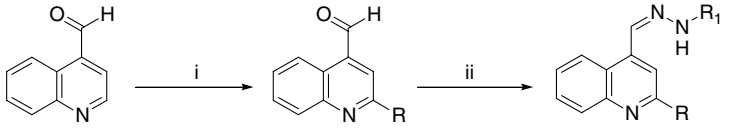
In vitro activities of the synthesized derivatives (Series1–4) against *M. tuberculosis H37Rv* strains (ATCC 27294, susceptible both to rifampicin and isoniazid) were initially carried out using the Microplate Alamar Blue Assay (MABA) at a concentration of 6.25 μg/mL.[12] Compounds exhibiting fluorescence were then tested in the BACTEC 460 radiometric system[9,13] and the % inhibition is summarized in Tables 1 and 2. Compounds demonstrating ⩾90% inhibition at 6.25 μg/mL in the primary screen were also tested at the lower concentration of 3.125 μg/mL to determine MIC value that is defined as the minimum concentration exhibiting 99% inhibition. Isoniazid (99% inhibition, MIC = 1 μg/mL) was included, as a standard drug, for comparison.

The intermediates 2-(1-adamantyl)-4-quinolinecarbaldehyde (**5**) and 2-cyclohexyl-4-quinolinecarbaldehyde (**6**) displayed weak activity with 36% and 26% inhibition, respectively, at 6.25 μg/mL against the drug-sensitive TB strain.

None of the *N*-(alkyl)-*N*′-quinolin-4-ylmethylene hydrazines **3a**–**f** (Series 1) was found active; only compound (**3e**) exhibited weak inhibition (26%) at 6.25 μg/mL (Table 1). Interestingly, derivatives **3a**–**c** bearing fluorophenyl hydrazine group were found inactive. This is presumably due to the inductive and resonance effects of fluorine on the quinoline ring basicity. While,

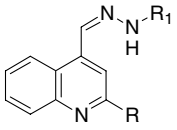
Table 1. In vitro antimycobacterial activity data of *N*-(alkyl)-*N*′-quinolin-4-ylmethylene-hydrazines/hydrazides **3a**–**f** (Series 1) and *N*-(alkyl)-*N*′-quinolin-2-ylmethylene-hydrazines/hydrazides **4a**–**f** (Series 2) against drug-sensitive strain of *Mycobacterium tuberculosis H37Rv*

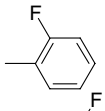
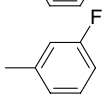
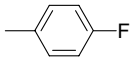
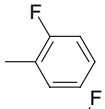
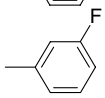
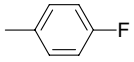
Compound	R	Test concn (μg/mL)	% inhibition
			
	Series 1		
3a		6.25	0
3b		6.25	0
3c		6.25	0
3d	CHO	6.25	0
3e	$COCH_3$	6.25	26
3f		6.25	0
			
	Series 2		
4a		3.125	99
4b		6.25	97
4c		6.25	94
4d	CHO	6.25	12
4e	$COCH_3$	6.25	5
4f		6.25	3



Scheme 2. Reagents and conditions: (i) 1-adamatanecarboxylic acid or cyclohexanecarboxylic acid, $AgNO_3$, $(NH_4)_2S_2O_8$, CH_3CN, 10% H_2SO_4, 30 min, rt; (ii) R_1NHNH_2, abs EtOH, 80 °C, 8 h.

Table 2. In vitro antimycobacterial activity data of *N*-(2-adamantan-1-yl-quinolin-4-yl-methylene)-N′-substituted hydrazines/hydrazides **7a–f** (Series 3) and *N*-(2-cyclohexyl-quinolin-4-yl-methylene)-N′-substituted hydrazines/hydrazides **8a–f** (Series 4) against drug-sensitive strain of *Mycobacterium tuberculosis H37Rv*



Compound	R	R_1	Test concn (μg/mL)	% inhibition
7a	Adamantan-1-yl		6.25	93
7b	Adamantan-1-yl		6.25	94
7c	Adamantan-1-yl		3.125	99
7d	Adamantan-1-yl	CHO	6.25	98
7e	Adamantan-1-yl	$COCH_3$	6.25	0
7f	Adamantan-1-yl		6.25	0
8a	*c*-C_6H_{11}		3.125	99
8b	*c*-C_6H_{11}		6.25	99
8c	*c*-C_6H_{11}		6.25	0
8d	*c*-C_6H_{11}	CHO	6.25	0
8e	*c*-C_6H_{11}l	$COCH_3$	6.25	0
8f	*c*-C_6H_{11}		6.25	0

compounds **4a–c** from the *N*-(alkyl)-*N*′-quinolin-2-ylmethylene-hydrazines (Series 2) showed promising results and inhibited the growth of mycobacteria by 94% at 6.25 μg/mL (Table 1). The most active compound of this series, *N*-(2-fluorophenyl)-*N*′-quinolin-2-ylmethylene-hydrazine (**4a**), was a promising inhibitor of *M. tuberculosis H37Rv* and displayed 99% inhibition at a lower dose of 3.125 μg/mL (Table 1). In agreement with our earlier observation,[6–9] placement of a cycloalkyl group like 1-adamantyl or cyclohexyl at the C-2 position in 4-quinolinecarbaldehyde derivatives led to a considerable improvement in anti-TB activity. For example, derivatives **7a–d** from the *N*-(2-adamantan-1-yl-quinolin-4-yl-methylene)-N′-substituted hydrazines/hydrazides (Series 3) inhibited the growth of drug-sensitive *M. tuberculosis H37Rv* by $\geqslant$93% at 6.25 μg/mL. The most active compound of this series, *N*-(2-adamantan-1-yl-quinolin-4-ylmethylene)-*N*′-(4-fluorophenyl)hydrazine (**7c**), also exhibited 99% inhibition at 3.125 μg/mL (Table 2).

Finally, derivatives **8a** and **8b** from the *N*-(2-cyclohexyl-quinolin-4-yl-methylene)-N′-substituted hydrazines/hydrazides (Series 4) inhibited the growth of mycobacteria by 99% at 6.25 μg/mL (Table 2). The most active compound *N*-(2-cyclohexyl-quinolin-4-ylmethylene)-*N*′-(2-fluorophenyl)hydrazine (**8a**) of the series also displayed 99% inhibition at 3.125 μg/mL (Table 2). These results indicate that (fluorophenyl)hydrazine/hydrazide derivatives of 2-(1-adamantyl)- and 2-cyclohexyl-4-quinolinecarbaldehydes exhibit anti-TB activity; while derivatives containing phenyl and aliphatic hydrazine/hydrazide groups were inactive. The only exception was formic acid (2-adamantan-1-yl-quinolin-4-ylmethylene)hydrazide (**7d**) (Series 3) which displayed 98% inhibition at 6.25 μg/mL (Table 2).

4. 3D-QSAR study

4.1. Methodology

The molecules **4a–c**, **7a–d**, and **8a** and **8b** have been classified as 'active' and remaining fifteen as 'inactive.' All the active molecules have pIC_{50} values above 5.85 log units. The pIC_{50} values were calculated as follows:[14]

$$pIC_{50} = -\log c + \text{logit}$$

where c is molar concentration = concentration (μg/mL) × 0.001/(molecular weight) and logit = log [% inhibition/(100 − % inhibition)].

The binary classification as active or inactive (1 and 0) has been used for the QSAR study. The molecules were built with the '*Sketch*' module of *Sybyl 7.1*(Tripos Inc., USA) installed on a Pentium PIV PC running under the Red Hat Enterprise WS 2.3 OS. These were then energy minimized by Powell's method using the MMFF94[15] force field with a distance-dependent dielectric term. The minimization was terminated when the gradient reached 0.5 kcal/mol/Å. Each structure was then subjected to molecular dynamics (MD) simulation, where it was heated to 700 K for 1 ps and annealed slowly to 200 K in steps of 100 K for 1 ps at each temperature, with a step size of 1 fs, and snapshots captured every 5 fs. The lowest energy structure from the MD trajectory was sent through a final round of minimization, which was carried out with the same criteria as mentioned above. Molecule **7c** was chosen as the template on which other molecules were aligned to it using the *Database Alignment* method in *Sybyl*. The aligned molecules were considered for the QSAR study. All descriptor calculations and statistical analysis were carried out using *TSAR 3.3* (Accelrys Inc., USA) for Windows OS.

4.2. Descriptor calculations

Molar refractivity (MR) and lipophilicity ($\log P$) of the molecules were calculated by the 'sum of atomic partial values' method as implemented in *TSAR 3.3*. Partial atomic charges were calculated at the AM1 level of theory as implemented in the *Vamp* module of *TSAR 3.3*. The similarity indices of molecules in terms of atomic charges, shape, lipophilicity, molar refractivity, and overall similarity were calculated using the *Asp* (Automated Similarity Package) module of *TSAR 3.3*. The similarity values provide a set of 3D-QSAR descriptors.[16] The *Asp*-similarity, both 'individual similarity indices' and 'combined similarity index', was calculated across the 24 molecules as the 'Carbo molecular similarity index'[17] for the molecules. The 'combined similarity index' was calculated for four different combinations: (i) charge and shape; (ii) charge, shape, and $\log P$; (iii) charge, shape and MR; and (iv) charge, shape, $\log P$, and MR.

4.3. Statistics

The standard data reduction technique of 'Principal Component Analysis' (PCA) was used to reduce the 24 columns of descriptors to the principal components that contain as much of the original information as possible. The data were scaled using mean/SD before PCA. The first three PCA vectors were added to the study table containing the binary representation of the activity of the molecules. 'Stepping discriminant analysis'[18] (SDA) was carried out on the binary activity data and the first three principal components PC1, PC2, and PC3. A graph of PC2 versus PC3 was then constructed and analyzed (vide infra).

4.4. Results and discussion

PCA is a method that is used to reduce a large number of variables to a smaller number without losing vital information, the original variables being transformed into a new orthogonal set of linear combinations. Each principal component (PC) is a combination of the original variables, defined by the PC loading coefficients. The first PC always explains the greatest variance, with decreasing account by the successive PCs. Each PC has an associated eigenvalue, which shows how much of the variance of the original data set is explained by that PC. The results of the PCA study on the four descriptors sets are shown in Table 3. In all cases, the first three PCs explain ~95–97 % of the total variance, hence, only the first 3 PCs were considered for analysis. Inclusion of MR or $\log P$ or both into the calculation of 'combined similarity index' did not improve the statistics significantly.

In the next step, the binary activity data were subjected to SDA with the first three PCs as the *X*-variables. Discriminant analysis is a technique that aims to separate two or more classes of compounds using a number of explanatory variables. *TSAR 3.3* uses the Mahalanobis[18] distance discrimination algorithm. A stepwise procedure was used to select a subset of the explanatory variables and optimize the classification rule. The entering variable, at each stage, is the one that gives the greatest increase in the total Mahalanobis distance between all pairs of class centers of all variables under consideration. The stepwise selection of explanatory variables ends when no variable gives more than 5% increase in the total Mahalanobis distance sum between class centers and the best variable reduces the total number of well-classified points. The results of SDA are shown in Table 4.

The analysis of SDA reveals that the variables PC2 and PC3 enter the model in the first two steps of stepping and offer significant classification of the data, whereas the variable PC1 never enters the model. The model constructed with the Similarity Index,

Table 3. Total variance explained and eigenvalues of the first three components from PC analysis of four sets of descriptors

	Combined similarity (charge, shape)		Combined similarity (charge, shape, log *P*)		Combined similarity (charge, shape, MR)		Combined similarity (charge, shape, MR, log *P*)	
	Total variance	Eigen value	Total variance	Eigen value	Total variance	Eigen value	Total variance	Eigen value
PC1	64	15.3	63	15.1	64	15.3	63	15.1
PC2	88	5.8	87	5.8	88	5.7	85	5.7
PC3	95	1.8	95	2.0	95	1.9	97	2.0

Table 4. The statistics of stepping discriminant analysis using the first three principal components

	Active predicted active	Active predicted active (CV)[a]	Inactive predicted inactive	Inactive predicted inactive (CV)[a]	Confidence for active	Confidence for inactive	Overall confidence
Combined similarity (charge, shape)	9	9	11	11	1.0	0.73	0.80
Combined similarity (charge, shape, log *P*)	9	9	11	11	1.0	0.73	0.80
Combined similarity (charge, shape, MR)	9	9	11	11	1.0	0.73	0.80
Combined similarity (charge, shape, MR, log *P*)	9	8	11	11	0.89	0.73	0.76

[a] Results of the cross-validation run.

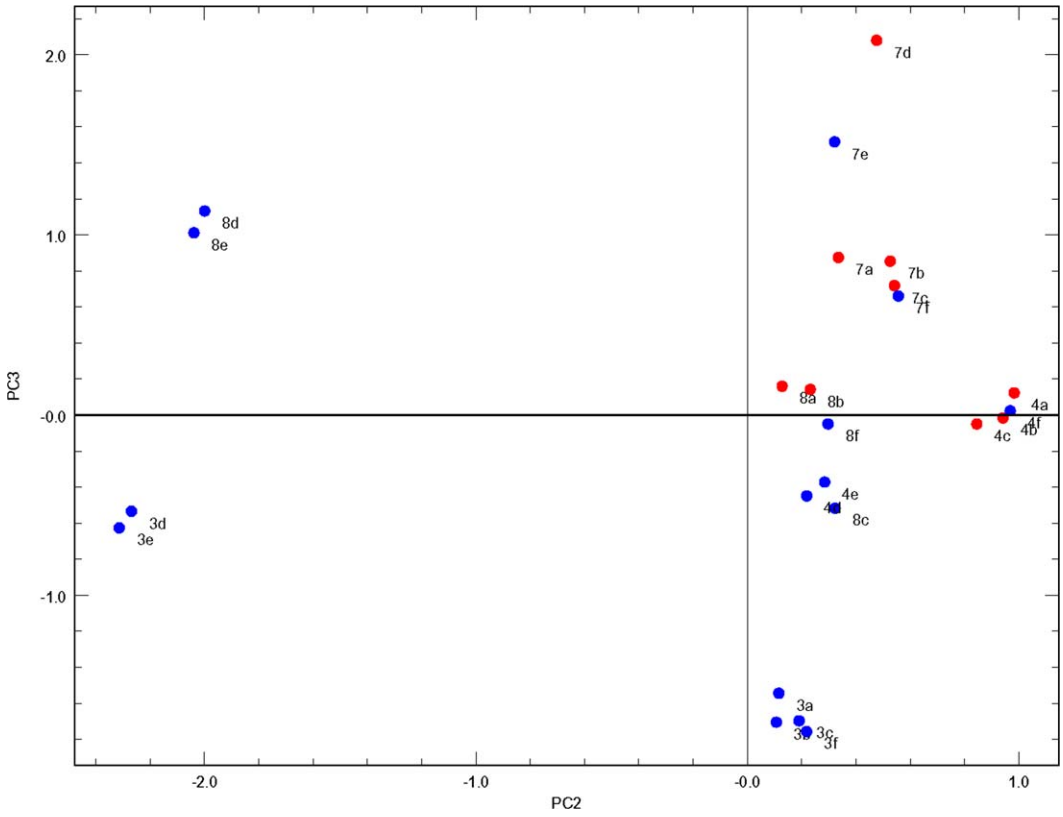


Figure 3. Plot of PC2 versus PC3 derived from the PCA of combined similarity descriptors calculated using charge and shape properties. The data points in red represent 'active' and those in blue represent 'inactive' molecules. The 'active' molecules are clustered in a small region.

calculated using charge and shape, shows a good classification of 'active' and 'inactive' molecules. The inclusion of MR and log *P*, individually or together, did not improve the statistics. All nine 'active' molecules were predicted correctly. Of the 15 'inactive' molecules, 11 were predicted correctly, but molecules **7e**, **7f**, **8f**, and **4f** have been predicted as 'active.' A graph of PC2 versus PC3 for this model is shown in Figure 3. All 'actives' cluster together in a small region, except for molecule **7d**. In this cluster of 'active' molecules, there exist three 'inactive' molecules **7f**, **8f**, and **4f**. The confidence for correctly predicting the 'active' molecule is 1.0, for 'inactive' molecules is 0.73, and the overall confidence is 0.80 (Table 4). In the present dataset of the nine 'active' and fifteen 'inactive' molecules, assigning each molecule at random should give a probability of 0.375 and 0.625 for the compounds correctly classified as 'active' and 'inactive,' respectively. The confidence estimate obtained by SDA is higher than that obtained by random calculations. Thus, the 'combined similarity index' calculated based on charge (electrostatic) and shape (steric) was able to classify the molecules into 'active' and 'inactive' classes with a high level of statistical confidence.

5. Conclusions

In conclusion, we have designed, synthesized, and evaluated ring-substituted-2/4-quinolinecarbaldehyde derivatives as inhibitors of *M. tuberculosis H37Rv*. This study was focused on the manipulation at the C-2 and C-4 positions of 2/4-quinolinecarbaldehydes and at the C-4 position of 2-(adamantan-1-yl)- and 2-cyclohexyl-4-quinolinecarbaldehydes. The study resulted in the identification of compounds **4a**, **7c**, and **8a** as promising inhibitors of *M. tuberculosis*. It is also clear that placement of a fluorine and its inductive and resonance effects on the basicity of ring-substituted quinolines led to a significant change in biological activity. All compounds were synthe-

sized in good yield using inexpensive starting materials in 1–2 overall steps thereby indicating their importance as the lead compounds in anti-tuberculosis drug discovery and development due to the poor demographic profile of the TB-patients. In an attempt to understand the essential structural requirements for anti-tuberculosis activity, we have performed a 3D-QSAR analysis of the synthesized compounds. Molecular modeling studies have thrown some insight into the observed SAR profile. For the present quinoline dataset, the similarity index based on electrostatic and steric features of the molecules, combined with PCA and SDA, is able to classify them as active or inactive within the limits of statistical significance. This strategy represents a promising approach for the discovery and development of the new ring-substituted quinoline compounds effective for the treatment of TB.

6. Experimental

Melting points were recorded on a Mettler DSC 851 instrument or a capillary melting point apparatus and are uncorrected. ^{1}H spectra were recorded on a 300 MHz Bruker FT-NMR (Avance DPX300) spectrometer using tetramethylsilane as internal standard and the chemical shifts are reported in δ units. Mass spectra were recorded on a HRMS (Finnigan Mat LCQ) spectrometer using ESI mode. Elemental analyses were carried out on an Elementar Vario EL spectrometer. Chromatographic purifications were carried out with silica gel 60 (230–400 mesh) and TLC (silica gel) was done on silica gel coated (Merck Kiesel 60 F_{254}, 0.2 mm thickness) sheets. All chemicals were purchased from Aldrich Chemical Ltd (Milwaukee, WI, USA). Solvents used for the chemical synthesis were acquired from commercial sources, were of analytical grade, and used without further purification unless otherwise stated.

6.1. General method for the synthesis of *N*-(alkyl)-*N*′-quinolin-4-yl-methylene-hydrazines/hydrazides 3a–f and *N*-(alkyl)-*N*′-quinolin-2-yl-methylene-hydrazines/hydrazides 4a–f

To a solution of 4-quinolinecarbaldehyde or 2-quinolinecarbaldehyde (**1** or **2**, 0.1 g, 0.63 mmol) in abs ethyl alcohol (10 mL), the aromatic or aliphatic hydrazine (0.75 mmol) was added, and the reaction mixture heated at 80 °C for 8 h. The reaction mixture was cooled to ambient temperature, and solvent removed to afford the crude product. Recrystallization of the crude product with 95% ethyl alcohol afforded compounds **3a**–**f** and **4a**–**f**.

6.1.1. *N*-(2-Fluorophenyl)-*N*′-quinolin-4-ylmethylene-hydrazine (3a). Yield: 74%; mp: 182–184 °C (dec); ^{1}H NMR ($CDCl_3$): δ 8.92 (d, 1H, J = 4.1 Hz), 8.60 (d, 1H, J = 8.2 Hz), 8.38 (s, 1H), 8.16 (d, 1H, J = 8.2 Hz), 7.70 (m, 4H), 7.15 (m, 1H), 7.05 (m, 1H), 6.88 (m, 1H); ESI-MS: *m*/*z* 266 (M+1). Anal. Calcd for $C_{16}H_{12}FN_3$ (265.3): C, 72.44; H, 4.56; N, 15.84. Found: C, 72.38; H, 4.54; N, 15.85.

6.1.2. *N*-(3-Fluorophenyl)-*N*′-quinolin-4-ylmethylene-hydrazine (3b). Yield: 55%; mp: 178–180 °C (dec); ^{1}H NMR ($CDCl_3$): δ 9.05 (br s, 1H), 8.72 (d, 1H, J = 4.5 Hz), 8.35 (d, 1H, J = 8.4 Hz), 8.07 (m, 2H), 7.62 (m, 1H), 7.46 (m, 1H), 7.30 (m, 2H), 7.17 (m, 2H), 6.94 (m, 1H); ESI-MS: *m*/*z* 266 (M+1). Anal. Calcd for $C_{16}H_{12}FN_3$ (265.3): C, 72.44; H, 4.56; N, 15.84. Found: C, 72.41; H, 4.51; N, 15.81.

6.1.3. *N*-(4-Fluorophenyl)-*N*′-quinolin-4-ylmethylene-hydrazine (3c). Yield: 58%; mp: 169–171 °C (dec); ^{1}H NMR ($CDCl_3$): δ 8.91 (d, 1H, J = 4.4 Hz), 8.57 (d, 1H, J = 7.6 Hz), 8.27 (s, 1H), 8.15 (d, 1H, J = 7.5 Hz), 7.68 (m, 3H), 7.41 (m, 2H), 7.06 (m, 2H); ESI-MS: *m*/*z* 266 (M+1). Anal. Calcd for $C_{16}H_{12}FN_3$ (265.3): C, 72.44; H, 4.56; N, 15.84. Found: C, 72.39; H, 4.52; N, 15.82.

6.1.4. Formic acid quinolin-4-ylmethylene-hydrazide (3d). Yield: 60%; mp: 178–180 °C (dec); ^{1}H NMR ($CDCl_3$): δ 10.12 (s, 1H), 8.99 (d, 1H, J = 4.7 Hz), 8.51 (m, 2H), 8.19 (d, 1H, J = 8.4 Hz), 7.78 (m, 2H), 7.66 (m, 1H); ESI-MS: *m*/*z* 200 (M+1). Anal. Calcd for $C_{11}H_9N_3O$ (199.2): C, 66.32; H, 4.55; N, 21.09; found: C, 66.31; H, 4.56; N, 21.01.

6.1.5. Acetic acid quinolin-4-ylmethylene-hydrazide (3e). Yield: 65%; mp: 172–174 °C (dec); ^{1}H NMR ($CDCl_3$): δ 8.98 (d, 1H, J = 4.5 Hz), 8.56 (d, 1H, J = 8.6 Hz), 8.41 (s, 1H), 8.19 (d, 1H, J = 8.3 Hz), 7.77 (m, 2H), 7.65 (m, 1H), 2.49 (s, 3H); ESI-MS: *m*/*z* 214 (M+1). Anal. Calcd for $C_{12}H_{11}N_3O$ (213.2): C, 67.59; H, 5.20; N, 19.71. Found: C, 67.53; H, 5.18; N, 19.77.

6.1.6. *N*-Phenyl-*N*′-quinolin-4-ylmethylene-hydrazine (3f). Yield: 60%; mp: 167–169 °C (dec); ^{1}H NMR ($CDCl_3$): δ 8.91 (d, 1H, J = 4.7 Hz), 8.55 (d, 1H, J = 8.3 Hz), 8.35 (s, 1H), 8.15 (d, 1H, J = 8.4 Hz), 7.74 (m, 2H), 7.63 (m, 1H), 7.23 (m, 5H); ESI-MS: *m*/*z* 248 (M+1). Anal. Calcd for $C_{16}H_{13}N_3$ (247.3): C, 77.71; H, 5.30; N, 16.99. Found: C, 77.65; H, 5.31; N, 17.03.

6.1.7. *N*-(2-Fluorophenyl)-*N*′-quinolin-2-ylmethylene-hydrazine (4a). Yield: 63%; mp: 180–182 °C (dec); ^{1}H NMR ($CDCl_3$): δ 8.72 (br s, 1H), 8.21 (m, 3H), 8.07 (d, 1H, J = 8.1 Hz), 7.78 (m, 2H), 7.57 (m, 1H), 7.43 (d, 1H, J = 8.5 Hz), 7.06 (m, 2H), 6.90 (d, 1H, J = 6.7 Hz); ESI-MS: *m*/*z* 266 (M+1). Anal. Calcd for $C_{16}H_{12}FN_3$ (265.2): C, 72.44; H, 4.56; N, 15.84. Found: C, 72.36; H, 4.53; N, 15.81.

6.1.8. *N*-(3-Fluorophenyl)-*N*′-quinolin-2-ylmethylene-hydrazine (4b). Yield: 65%; mp: 175–177 °C (dec); ^{1}H NMR ($CDCl_3$): δ 8.18 (m, 2H), 8.09 (m, 2H), 7.81 (d, 1H, J = 8.2 Hz), 7.73 (m, 1H), 7.56 (m, 1H), 7.10 (m, 3H), 6.87 (m, 1H); ESI-MS: *m*/*z* 266 (M+1). Anal. Calcd for $C_{16}H_{12}FN_3$ (265.3): C, 72.44; H, 4.56; N, 15.84. Found: C, 72.37; H, 4.54; N, 15.80.

6.1.9. *N*-(4-Fluorophenyl)-*N*′-quinolin-2-ylmethylene-hydrazine (4c). Yield: 70%; mp: 167–169 °C (dec); ^{1}H NMR ($CDCl_3$): δ 8.17 (m, 2H), 8.04 (d, 1H, J = 8.7 Hz), 7.96 (s, 1H), 7.81 (d, 1H, J = 7.9 Hz), 7.70

(m, 1H), 7.52 (m, 1H), 7.03 (d, 2H, J = 10.9 Hz), 6.86 (d, 2H, J = 8.1 Hz); ESI-MS: m/z 266 (M+1). Anal. Calcd for $C_{16}H_{12}FN_3$ (265.3): C, 72.44; H, 4.56; N, 15.84. Found: C, 72.39; H, 4.51; N, 15.80.

6.1.10. Formic acid quinolin-2-ylmethylene-hydrazide (4d). Yield: 75%; mp: 178–180 °C (dec); ^{1}H NMR ($CDCl_3$): δ 8.89 (s, 1H), 8.21 (d, 1H, J = 8.4 Hz), 8.07 (m, 3H), 7.85 (d, 1H, J = 8.1 Hz), 7.75 (m, 1H), 7.61 (m, 1H); ESI-MS: m/z 200 (M+1). Anal. Calcd for $C_{11}H_9N_3O$ (199.2): C, 66.32; H, 4.55; N, 21.09. Found: C, 66.28; H, 4.51; N, 21.05.

6.1.11. Acetic acid quinolin-2-ylmethylene-hydrazide (4e). Yield: 80%; mp: 187–189 °C (dec); ^{1}H NMR ($CDCl_3$): δ 9.15 (br s, 1H), 8.18 (d, 1H, J = 8.5 Hz), 8.06 (m, 3H), 7.84 (d, 1H, J = 8.1 Hz), 7.75 (m, 1H), 7.60 (m, 1H), 2.45 (s, 3H); ESI-MS: m/z 214 (M+1). Anal. Calcd for $C_{12}H_{11}N_3O$ (213.2): C, 67.59; H, 5.20; N, 19.71. Found: C, 67.63; H, 5.24; N, 19.74.

6.1.12. *N*-Phenyl-*N*′-quinolin-2-ylmethylene-hydrazine (4f). Yield: 70%; mp: 174–176 °C (dec); ^{1}H NMR ($CDCl_3$): δ 8.18 (m, 3H), 8.07 (d, 1H, J = 8. 4 Hz), 7.77 (m, 2H), 7.56 (m, 1H), 7.33 (m, 5H); ESI-MS: m/z 248 (M+1). Anal. Calcd for $C_{16}H_{13}N_3$ (247.3): C, 77.71; H, 5.30; N, 16.99. Found: C, 77.68; H, 5.34; N, 17.02.

6.2. General method for the synthesis of 2-alkyl-4-quinolinecarbaldehydes 5 and 6

To a mixture of 4-quinolinecarbaldehyde (**1**, 2.0 g, 13 mmol), $AgNO_3$ (1.2 g, 7 mmol), alkylcarboxylic acid (52 mmol) in CH_3CN (8 mL), and 10% H_2SO_4 (20 mL) was added. The reaction mixture was stirred at ambient temperature and a freshly prepared solution of ammonium persulfate (40 mmol) in water (10 mL) was added dropwise in 2 min. After stirring for additional 30 min, the reaction was terminated by pouring it onto ice. The resulting mixture was made alkaline with 25% NH_4OH solution and extracted with ethyl acetate (3× 50 mL). The combined extracts were washed with brine solution (2× 25 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to afford oil, which upon chromatographic purification over silica gel (230–400 mesh) using EtOAc/hexanes (8:92) gave compounds **5** and **6**.

6.2.1. 2-(1-Adamantyl)-4-quinolinecarbaldehyde (5). Yield: 35%; semi-solid; ^{1}H NMR ($CDCl_3$): δ 9.92 (s, 1H), 8.94 (d, 1H, J = 8.4 Hz), 8.16 (d, 1H, J = 8.3 Hz), 7.88 (s, 1H), 7.76 (m, 1H), 7.64 (m, 1H), 1.88 (m, 15H); ESI-MS: m/z 292 (M+1). Anal. Calcd for $C_{20}H_{21}NO$ (291.4): C, 82.44; H, 7.26; N, 4.81. Found: C, 82.38; H, 7.21; N, 4.76.

6.2.2. 2-Cyclohexyl-4-quinolinecarbaldehyde (6). Yield: 25%; semi-solid; ^{1}H NMR ($CDCl_3$): δ 10.43 (s, 1H), 8.89 (d, 1H, J = 8.4 Hz), 8.11 (d, 1H, J = 8.4 Hz), 7.70 (m, 2H), 7.58 (m, 1H), 2.98 (m, 1H), 1.52 (m, 10H); ESI-MS: m/z 240 (M+1). Anal. Calcd for $C_{16}H_{17}NO$ (239.3): C, 80.30; H, 7.16; N, 5.85. Found: C, 80.35; H, 7.18; N, 5.88.

6.3. General method for the synthesis of *N*-(2-adamantan-1-yl-quinolin-4-yl-methylene)-N′-substituted hydrazines/hydrazides 7a–f and *N*-(2-cyclohexyl-quinolin-4-yl-methylene)-N′-substituted hydrazines/hydrazides 8a–f

To a solution of 2-alkyl-4-quinolinecarbaldehyde (**5** and **6**, 0.35 mmol) in abs ethyl alcohol (10 mL), requisite aromatic or aliphatic hydrazine (0.42 mmol) was added, and the reaction mixture heated at 80 °C for 8 h. The reaction mixture was cooled to ambient temperature and the solvent evaporated under reduced pressure. The resulting residue was diluted with ethyl acetate (50 mL), washed with water (2× 25 mL) followed with brine solution (2× 25 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to afford oily residue, which upon column chromatographic purification over neutral alumina using EtOAc/hexanes (8:92) gave compounds **7a**–**f** and **8a**–**f**.

6.3.1. *N*-(2-Adamantan-1-yl-quinolin-4-ylmethylene)-*N*′-(2-fluorophenyl)hydrazine (7a). Yield: 67%; mp: 172–174 °C (dec); ^{1}H NMR ($CDCl_3$): δ 8.61 (d, 1H, J = 8.4 Hz), 8.36 (s, 1H), 8.11 (d, 1H, J = 8.5 Hz), 7.78 (s, 1H), 7.69 (m, 2H), 7.57 (m, 1H), 7.19 (m, 1H), 7.09 (m, 1H), 6.89 (m, 1H), 1.88 (m, 15H); ESI-MS: m/z 400 (M+1). Anal. Calcd for $C_{26}H_{26}FN_3$ (399.5): C, 78.17; H, 6.56; N, 10.52. Found: C, 78.13; H, 6.51; N, 10.57.

6.3.2. *N*-(2-Adamantan-1-yl-quinolin-4-ylmethylene)-*N*′-(3-fluorophenyl)hydrazine (7b). Yield: 53%; semi-solid; ^{1}H NMR ($CDCl_3$): δ 8.56 (br s, 1H), 8.46 (d, 1H, J = 8.5 Hz), 8.21 (s, 1H), 8.15 (d, 1H, J = 8.4 Hz), 7.73 (s, 1H), 7.65 (m, 1H), 7.51 (m, 2H), 6.97 (m, 1H), 6.87 (d, 1H), 6.61 (m, 1H), 1.88 (m, 15H); ESI-MS: m/z 400 (M+1). Anal. Calcd for $C_{26}H_{26}FN_3$ (399.5): C, 78.17; H, 6.56; N, 10.52 Found: C, 78.24; H, 6.51; N, 10.56.

6.3.3. *N*-(2-Adamantan-1-yl-quinolin-4-ylmethylene)-*N*′-(4-fluorophenyl)hydrazine (7c). Yield: 63%; semi-solid; ^{1}H NMR ($CDCl_3$): δ 8.45 (d, 1H), 8.24 (s, 1H), 8.14 (d, 1H, J = 8.4 Hz), 7.74 (s, 1H), 7.70 (m, 1H), 7.51 (m, 1H), 7.13 (m, 2H), 7.02 (m, 2H), 1.88 (m, 15H); ESI-MS: m/z 400 (M+1). Anal. Calcd for $C_{26}H_{26}FN_3$ (399.5): C, 78.17; H, 6.56; N, 10.52. Found: C, 78.17; H, 6.61; N, 10.57.

6.3.4. Formic acid (2-adamantan-1-yl-quinolin-4-ylmethylene)hydrazide (7d). Yield: 67%; mp: 170–172 °C (dec); ^{1}H NMR ($CDCl_3$): δ 9.92 (s, 1H), 8.96 (d, 1H, J = 10.3 Hz), 8.46 (s, 1H), 8.13 (d, 1H, J = 8.2 Hz), 7.79 (s, 1H), 7.72 (m, 1H), 7.57 (m, 1H), 1.88 (m, 15H); ESI-MS: m/z 334 (M+1). Anal. Calcd for $C_{21}H_{23}N_3O$ (333.4): C, 75.65; H, 6.95; N, 12.60; found: C, 75.59; H, 6.88; N, 12.57.

6.3.5. Acetic acid (2-adamantan-1-yl-quinolin-4-ylmethylene)hydrazide (7e). Yield: 66%; mp: 158–160 °C (dec); ^{1}H NMR ($CDCl_3$): δ 9.86 (br s, 1H), 8.59 (d, 1H, J = 8.2 Hz), 8.34 (s, 1H), 8.13 (d, 1H, J = 8.3 Hz), 7.71 (m, 2H), 7.56 (m, 1H), 2.51 (s, 3H), 1.88 (m, 15H); ESI-MS: m/z 348 (M+1). Anal. Calcd for $C_{22}H_{25}N_3O$ (347.5): C, 76.05; H, 7.25; N, 12.09. Found: C, 76.11; H, 7.32; N, 12.17.

6.3.6. *N*-(2-Adamantan-1-yl-quinolin-4-ylmethylene)-*N*′-phenylhydrazine (7f). Yield: 60%; semi-solid; ^{1}H NMR ($CDCl_3$): δ 8.56 (br s, 1H), 8.49 (d, 1H, J = 8.5 Hz), 8.20 (m, 2H), 7.74 (s, 1H), 7.68 (m, 1H), 7.52 (m, 1H), 7.26 (m, 5H), 1.88 (m, 15H); ESI-MS: *m*/*z* 382 (M+1). Anal. Calcd for $C_{26}H_{27}N_3$ (381.5): C, 81.85; H, 7.13; N, 11.01. Found: C, 81.91; H, 7.19; N, 11.09.

6.3.7. *N*-(2-Cyclohexyl-quinolin-4-ylmethylene)-*N*′-(2-fluorophenyl)hydrazine (8a). Yield: 62%; semi-solid; ^{1}H NMR ($CDCl_3$): δ 8.56 (d, 1H), 8.35 (s, 1H), 8.11 (d, 1H, J = 8.3 Hz), 7.68 (m, 3H), 7.56 (m, 1H), 7.18 (m, 1H), 7.11 (m, 1H), 6.89 (m, 1H), 2.94 (m, 1H), 1.52 (m, 10H); ESI-MS: *m*/*z* 348 (M+1). Anal. Calcd for $C_{22}H_{22}FN_3$ (347.4): C, 76.05; H, 6.38; N, 12.09. Found: C, 76.09; H, 6.43; N, 12.13.

6.3.8. *N*-(2-Cyclohexyl-quinolin-4-ylmethylene)-*N*′-(3-fluorophenyl)hydrazine (8b). Yield: 61%; semi-solid; ^{1}H NMR ($CDCl_3$): δ 9.15 (br s, 1H), 8.44 (d, 1H, J = 8.3 Hz), 8.28 (s, 1H), 8.20 (d, 1H, J = 8.3 Hz), 7.73 (m, 2H), 7.54 (m, 1H) 7.34 (s, 1H), 7.09 (d, 1H, J = 10.8 Hz), 6.97 (d, 1H, J = 7.8 Hz), 6.71 (m, 1H), 3.03 (m, 1H), 1.52 (m, 10H); ESI-MS: *m*/*z* 348 (M+1). Anal. Calcd for $C_{22}H_{22}FN_3$ (347.4): C, 76.05; H, 6.38; N, 12.09. Found: C, 76.07; H, 6.44; N, 12.13.

6.3.9. *N*-(2-Cyclohexyl-quinolin-4-ylmethylene)-*N*′-(4-fluorophenyl)hydrazine (8c). Yield: 60%; semi-solid; ^{1}H NMR ($CDCl_3$): δ 8.37 (d, 1H, J = 7.0 Hz), 8.16 (s, 1H), 8.04 (d, 1H, J = 8.2 Hz), 7.59 (m, 1H), 7.54 (s, 1H), 7.43 (m, 1H), 7.07 (m, 2H), 6.96 (m, 2H), 2.86 (m, 1H), 1.52 (m, 10H); ESI-MS: *m*/*z* 348 (M+1). Anal. Calcd for $C_{22}H_{22}FN_3$ (347.4): C, 76.05; H, 6.38; N, 12.09; found: C, 76.01; H, 6.33; N, 12.06.

6.3.10. Formic acid (2-cyclohexyl-quinolin-4-ylmethylene)hydrazide (8d). Yield: 77%; semi-solid; ^{1}H NMR ($CDCl_3$): δ 8.40 (d, 1H, J = 8.3 Hz), 8.30 (s, 1H), 8.06 (d, 1H, J = 8.3 Hz), 7.67 (m, 1H), 7.57 (s, 1H), 7.49 (m, 1H), 2.91 (s, 1H), 1.52 (m, 10H); ESIMS: *m*/*z* 282 (M+1). Anal. Calcd for $C_{17}H_{19}N_3O$ (281.3): C, 72.57; H, 6.81; N, 14.94. Found: C, 72.52; H, 6.77; N, 14.91.

6.3.11. Acetic acid (2-cyclohexyl-quinolin-4-ylmethylene)hydrazide (8e). Yield: 65%; semi-solid; ^{1}H NMR ($CDCl_3$): δ 9.85 (br s, 1H), 8.53 (d, 1H, J = 8.3 Hz), 8.35 (s, 1H), 8.12 (d, 1H, J = 8.3 Hz), 7.73 (m, 1H), 7.59 (m, 2H), 2.96 (s, 1H), 2.50 (s, 3H), 1.52 (m, 10H); ESI-MS: *m*/*z* 296 (M+1). Anal. Calcd for $C_{18}H_{21}N_3O$ (295.4): C, 73.19; H, 7.17; N, 14.23. Found: C, 73.13; H, 7.13; N, 14.18.

6.3.12. *N*-(2-Cyclohexyl-quinolin-4-ylmethylene)-*N*′-phenylhydrazine (8f). Yield: 66%; semi-solid; ^{1}H NMR ($CDCl_3$): δ 8.37 (d, 1H, J = 8.2 Hz), 8.21 (m, 2H), 7.67 (m, 2H), 7.50 (m, 1H), 7.33 (m, 5H), 2.96 (m, 1H), 1.52 (m, 10H); ESI-MS: *m*/*z* 330 (M+1). Anal. Calcd for $C_{22}H_{23}N_3$ (329.2): C, 80.21; H, 7.04; N, 12.76. Found: C, 80.17; H, 7.03; N, 12.74.

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