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Substituted hydrazinecarbothioamide as potent antitubercular agents: Synthesis and quantitative structure–activity relationship (QSAR)

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ABSTRACT

A series of novel substituted hydrazinecarbothioamides was synthesized and evaluated for anti-TB activity. Three most active compounds viz. **1**, 6 and **12** were found to exhibit minimum inhibitory concentration (MIC) of 0.4 μg/mL, whereas four compounds viz. **3**, **5**, 10 and **11** showed comparatively lesser activity with MIC value of 0.8 μg/mL against *Mycobacterium tuberculosis* strain. A highly significant QSAR equation explaining 81.8% variance is described.

Tuberculosis (TB) is one of the respiratory diseases mainly caused by *Mycobacterium tuberculosis* (MTb).[1] World Health Organisation estimates that one-third of the world's population is infected by *M. tuberculosis*. Annually TB affects 8 million people and causes 2–3 million deaths around the world. The rising number of TB affected people is due to the use of immunosuppressive drugs or substance abuse or HIV/AIDS which is a serious threat to TB control and prevention.[2] The DOTS (Directly Observed Therapy, Short-course)[3] involve a 3 or 4 drug regimen comprising isoniazid, rifampin, pyrazinamide, and/or ethambutol for a minimum of 6 months. Moreover, the emergence of multi-drug resistant TB (MDR-TB), and the extensively drug resistant (XDR-TB) strains has led to the discovery and the development of new potent anti-TB drugs a global priority.[4]

MTb produces two series of structurally related peptidic siderophores known as the mycobactins and carboxymycobactins that vary by the appended lipid residue. Mycobactins have emerged as attractive targets for the development of anti-TB agent because of their critical role in the growth and virulence of MTb.[5–7] Thus the development of species resistance against anti-TB drugs in mycobacterium species are forcing for the development of novel drugs as anti-TB agents.

In view of the above and activity associated with hydrazinecarbothioamide,[8] we have designed a set of novel substituted hydrazinecarbothioamide using the state of art approaches (substructural approach based on the pattern recognition). These molecules have been synthesized and screened for their anti-TB activities. The common structure formula of the synthesized titles compounds is shown in Figure 1.

The general synthesis of the novel substituted hydrazinecarbothioamides is outlined in Scheme 1. The first step involves the reaction of hydrazine hydrate with carbon disulfide in the presence of KOH at 0–10 °C. The next step involves the dropwise addition of methyl iodide to the ice-cooled reaction mixture over nearly 2 h. During this process, the colour of the reaction mixture changes from white (colourless) to yellow which indicates the formation of intermediate I, methyl hydrazinecarbodithionate.[9]

The third step involves the addition of the substituted acetophenone to the solution of I in 2-propanol to afford the intermediate II.[10] The last step involves the reaction of the intermediate II with the substituted/unsubstituted amines to afford the title compounds.[11]

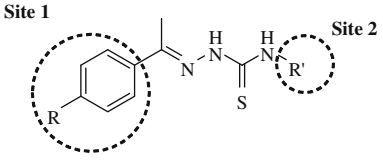

Figure 1. The common structural formula of the title compounds with encircled portions indicate the modification sites viz. site 1 and 2.

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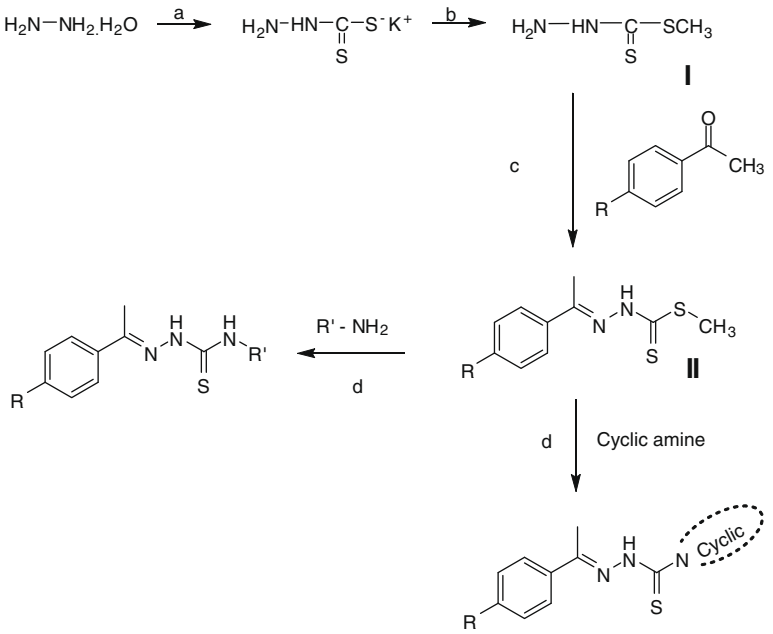


Scheme 1. Synthesis of substituted hydrazinecarbothioamides. Reagents and conditions: (a) CS_2 (3 mol), KOH (3 mol), 0–10 °C; (b) CH_3I (3 mol), rt; (c) 2-propanol, rt; (d) ethanol, rt.

The 'proportion method', described by McClachy[12] was followed for the determination of anti-TB activity. The synthesized title compounds exhibited good inhibition of mycobacterium species with minimum inhibitory concentration (MIC) ranging from 0.4 to 25 μg/mL (Table 1). Three most active compounds **1**, 6 and **12** exhibited MIC value of 0.4 μg/mL. The compounds **3**, **5**, 10 and **11** were found to be of comparatively lesser activity with MIC value of 0.8 μg/mL. All these compounds were having bromo group substituted to the phenyl ring (site 1, Fig. 1). The nitro substituted compounds exhibited MIC value of >3.12 μg/mL. The SAR analysis showed that the bromo substituted compound **12** (R = Br, MIC = 0.4 μg/mL) was about 7 times more potent than the nitro substituted compound **17** (R = NO_2, MIC = 3.12 μg/mL). Similarly, the compound **5** (R = Br, MIC = 0.8 μg/mL) was about 15 times more potent than the compound **21** (R = NO_2, MIC >12.5 μg/mL). Therefore, it can be concluded that the substitution with more hydrophobic/bulky groups in the site 1 may lead to more active compounds with lower MIC values.

Among the bromo substituted compounds (R = Br in site 1), the substitution of more electron withdrawing groups such as halogens, nitro etc. at the ortho position of the aromatic ring (site 2, Fig. 1) of provided compounds viz. **1**, **3**, **5**, **6**, **10**, 11 and **12** with comparatively better antitubercular activity (MIC <0.8 μg/mL).

In order to explore the important physicochemical and structural parameters responsible for the modulation of the anti-TB activities of the synthesized compounds, it appeared of interest to develop a linear regression quantitative-SAR (QSAR) model considering anti-TB activities (MIC) of a set of 14 compounds and a set of 454 descriptors generated using Dragon[13] software. The linear regression method implemented in the Systat (*v12*)[14] software was used to develop the quantitative relationship between dependent (*p*MIC) and the independent (454 Dragon descriptors) variables. Stepwise multi parameter regression analysis by forward elimination provided a set of three independent variables viz. BEHm6,[15] ARR[16] and G3u[17] whose definitions are illustrated in Table 2.

These three variables were found to be a good correlate for the *p*MIC values of the 14 compounds as anti-TB agents. The pearson correlation matrix is given below.

	pMIC	BEHm6	ARR	G3u
BEHm6	0.380	1.000		
ARR	0.332	−0.383	1.000	
G3u	−0.607	−0.070	0.122	1.000

This model was of good significance in terms of correlation (r) and standard error of estimate (SEE). The best model is illustrated in Eq. (1).

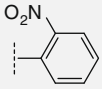
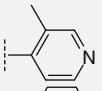
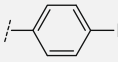
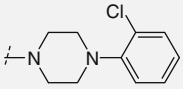
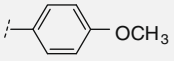
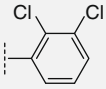
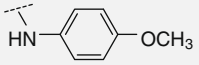
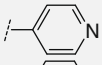
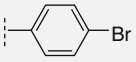
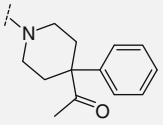
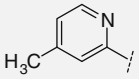
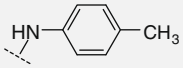
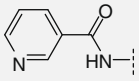
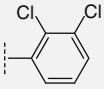
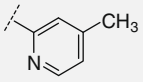
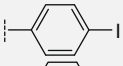
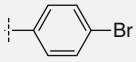
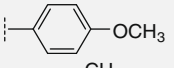
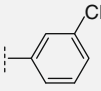
$$pMIC = 1.944(\pm 0.493)BEHm6 + 0.538(\pm 0.125)ARR - 26.977(\pm 5.701)G3u - 1.940 \quad (1)$$

$N = 14$; $r = 0.904$; $r^2 = 0.818$; $s = 0.206$; $F = 14.981$; $p = 0.0005$,where figures in parentheses denote the standard error of the regression coefficient, N is the number of compounds, r is the correlation coefficient, s is the standard deviation of the regression and F is the value of the Fisher's test for the overall significance of the equation. This model with ⩾99% statistical significance (probability $p = 0.0005$ explained 81.8% of the variance of the anti-TB activity of the 14 compounds. The positive correlation of *p*MIC with BEHm6 (sixth highest eigen value of burden matrix corresponding to atomic mass) and ARR (fraction of aromatic atoms) descriptors indicates their conducive effect towards inhibition of mycobacterium species. The negative correlation of *p*MIC with G3u (third component symmetry directional WHIM index) indicates its negative contribution towards inhibition of mycobacterium species.

The important descriptors included in the best QSAR equation along with the observed and predicted *p*MIC values of 14 compounds are given in Table 3. The 2D scatter plot between the observed and predicted *p*MIC values of the 14 compounds is shown in Figure 2.

In order to assess the predictive ability of the developed best QSAR model (equation 1), the inactive compounds viz. **2**, **9**, **14**, **16**, **18**, **20**–**22** with MIC values >12.5 μg/mL were used. The best model predicted all of these compounds inactive with MIC values >33 μg/mL. This analysis further proved the robustness of the best

Table 1
Structures and in vitro activities of the title compounds

Compd	R	R′	MIC[a]	Mp[b]
1	Br		0.4	121
2	Br		>12.5	104
3	Br		0.8	128
4	Br		3.12	145
5	Br		0.8	123
6	Br		0.4	123
7	Br		1.56	105
8	Br		3.12	135
9	Br		>12.5	90
10	Br		0.8	115
11	Br		0.8	125
12	Br		0.4	121
13	Br		1.56	215
14	Br		>12.5	ss[c]
15	Br		6.25	132
16	NO_2		>12.5	178
17	NO_2		3.12	170
18	NO_2		>12.5	ss
19	NO_2		6.25	129
20	NO_2		>12.5	158
21	NO_2		>12.5	134
22	NO_2		>12.5	157
INH			0.02	
ETHAMBUTOL			0.10	

[a] Minimum inhibitory concentration (μg/mL).
[b] Melting point (°C).
[c] Semi-solid.

Table 2
Definitions of the best three parameters

SN	Variable's name	Definition
1	BEHm6	Sixth highest eigen value of Burden matrix/weighted by atomic masses
2	ARR	Aromatic ratio
3	G3u	Third component symmetry directional WHIM index/ unweighted

Table 3
Dragon descriptors, observed (Obsd) and predicted (Pred.) anti-TB activities of the 14 compounds used in QSAR model development

Compd	Dragon descriptors			Anti-TB activity, pMIC (μg/mL)	
	BEHm6	ARR	G3u	Obsd	Pred.
1	3.141	0.667	0.162	0.398	0.334
3	3.141	0.636	0.164	0.097	0.280
4	3.238	0.483	0.172	−0.494	−0.285
5	3.022	0.609	0.174	0.097	−0.221
6	3.205	0.609	0.164	0.398	0.404
7	3.084	0.583	0.158	−0.193	−0.207
8	2.791	0.636	0.164	−0.494	−0.401
10	3.141	0.636	0.173	0.097	0.037
11	3.156	0.467	0.149	0.097	0.176
12	3.023	0.609	0.159	0.398	0.185
13	3.037	0.609	0.159	−0.193	−0.325
15	3.138	0.625	0.189	−0.796	−0.938
17	2.881	0.667	0.174	−0.494	−0.495
19	2.961	0.667	0.177	−0.796	−0.421

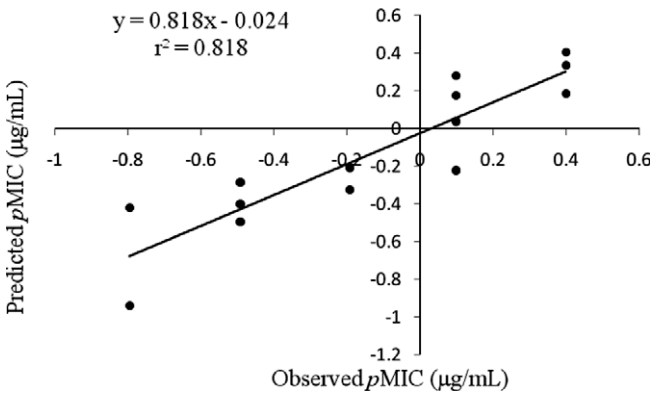


Figure 2. 2D scatter plot between the observed and predicted *p*MIC values of the 14 compounds.

QSAR model and provided a confidence in its utility in the prediction of the designed compounds prior to their synthesis and biological evaluation.

It can be concluded that the hydrazinecarbothioamide represent a promising bioactive core (substructure). The incorporation of the important groups at site 1 and site 2 (Fig. 1) has led to potent anti-TB compounds viz. **1**, **6** and **12** with MIC of 0.4 μg/mL. Compounds viz. **3**, **5**, **10** and **11** have been found to be of comparatively lesser activity with MIC value of 0.8 μg/mL. The above SAR and QSAR analyses comprising design and synthesis of anti-TB agents have provided vital informations for potential inhibition of the Mtb which may be useful in the design of the compounds with more potent anti-TB activities.

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9. *Synthesis of intermediate* I: The CS_2 (2.5 mL) was added drop-wise to a ice-cooled mixture of hydrazine hydrate (85%, 2 mL) in aqueous KOH (3 mol) and 2-propanol (10 mL) for 1–2 h at 0–10 °C. The stirring was continued for 1 h with precipitated yellow solid, followed by dropwise addition of iodomethane (2.5 mL) leading to the colourless (white) solution. The colourless mixture was stirred for an additional 1–2 h and the white precipitate was filtered and washed with cold water. The crude product was recrystallized with dichloromethane to afford colourless methyl hydrazinecarbodithionate, **I** (86.5%).
10. *Synthesis of methyl 2-(1-(4-bromophenyl) ethylidene) hydrazinecarbodithionate* (Intermediate II): The equimolar solution of methyl hydrazinecarbodithionate (I) and 4-bromo acetophenone in 2-propanol was stirred for about half an hour. The reaction mixture turned into yellow precipitate. This was stirred for additional 2–3 h and kept as such overnight. The separated crystals were filtered, washed with cold 2-propanol and air dried to yield the intermediate product II (59.2%).
11. *Synthesis of the title compound* **5**: The 4-methoxyaniline (1.23 g, 0.01 mol) was added to a warm solution of the methyl 2-(1-(4-bromophenyl)ethylidene)-hydrazinecarbodithioate (3 g, 0.01 mol) in warm ethanol. The reaction mixture was refluxed overnight. The resulted solid was filtered and recrystallized in absolute ethanol to yield the title compound **1** (94.5%). Chemical formula: $C_{16}H_{16}BrN_3OS$; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 1.95 (s, 3H), 2.13 (t, 4H), 7.33 (d, 4H), 7.00 (d, 3H); IR (KBr) cm^{-1}:676, 740, 1246, 2947,3188, 3451. Compound **6** (92%): Chemical formula: $C_{15}H_{12}BrCl_2N_3S$; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 6.36 (m, 7H), 8.89 (s, 1H), 9.70(s, 1H); IR (KBr) cm^{-1} 667, 772, 2927, 3245, 3451.
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