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Design, synthesis, and *in vitro* biological evaluation of novel benzimidazole tethered allylidenehydrazinylmethylthiazole derivatives as potent inhibitors of *Mycobacterium tuberculosis*[†]

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Tuberculosis (TB) has become one of the most significant public health problems in recent years. Antibiotic therapy remains the mainstay of TB control strategies, but the increasing resistance of mycobacterial species has heightened alarm, requiring the development of novel drugs in order to improve treatment outcomes. Here, as an effort to identify novel and effective antitubercular agents, we designed and synthesized a series of novel substituted benzimidazolallylidenehydrazinylmethylthiazole derivatives via a multi-component molecular hybridization approach with single molecular architecture. Our design strategy involved assembling the antitubercular pharmacophoric fragments benzimidazole, 2-aminothiazole and substituted α,β -unsaturated ketones via condensation reactions. All the newly synthesized compounds were fully characterized via NMR and mass spectral data and evaluated for *in vitro* biological activity against the H37Ra strain of *Mycobacterium tuberculosis*. From the biological evaluation data, we identified some effective compounds, of which **8g** and **7e** were the most active ones (both having MIC values of 2.5 $\mu\text{g mL}^{-1}$). In addition, compound **8g** exhibited a lower cytotoxicity profile. We conceive that compound **8g** may serve as a chemical probe of interest for further lead optimization studies with the general aim of developing novel and effective antitubercular agents.

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1. Introduction

Tuberculosis (TB) is one of the serious infectious diseases caused by *Mycobacterium tuberculosis* (Mtb) and becomes lethal when co-infected with HIV.^{1–3} According to recent reports by the World Health Organization (WHO), worldwide, 10.4 million new tuberculosis patients were identified in 2016, from which 1.67 (16.1%) million deaths were recorded.⁴ The majority of TB infected people can be cured with early diagnosis and proper treatment. Antibiotic therapy remains the mainstay of TB control strategies. However, the long duration

of treatment (at least 6 months), toxicity with low efficacy, and health burden are the main barriers regarding treating TB patients using drugs.⁵ In addition, the increasing resistance of *Mycobacterium* species to existing drugs has heightened alarm about TB in the international health community.⁶ In particular, the emergence of multi-drug resistant strains (MDR-TB), which are resistant to the first-line drugs isoniazid and rifampicin, and more recently the emergence of extensively drug-resistant strains (XDR-TB), which are MDR-TB strains also resistant to any fluoroquinolone drug and to at least one of the three injectable drugs used for TB treatment (amikacin, capreomycin and kanamycin), have complicated treatment outcomes.⁷ In 2016, around 600 000 new TB patients were diagnosed as having resistance to rifampicin (RRTB); among these, 490 000 patients had MDR-TB while 8000 patients had XDR-TB. The lack of new drugs as well as druggable targets, which could be utilized for developing more efficient anti-TB agents, are major hurdles to tackling this resistance.^{6,8–11} Clearly, there is a pressing need to identify and develop novel drug-based antimycobacterial therapies in order to improve treatment outcomes.⁸ However, the drug

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development pipeline in this regard remains woefully thin, with little chemical diversity.^{12–15}

In recent years, synthetic heterocyclic hybrid molecules have exhibited therapeutic potential against many diseases.^{16–19} Particularly, heterocyclic compounds bearing benzimidazole and aminothiazole moieties have shown a broad range of medicinal advantages, having demonstrated antitubercular, antihelminthic, antifungal, anticancer, and antiulcer activities.²⁰ Among the benzimidazole-derived heterocyclics, trisubstituted SB-P3G2 (**I**) and SB-P8B2 (**II**) have shown good activity against drug-resistant and susceptible strains of *Mtb*, and have also exhibited efficacy in a murine *Mtb* infection model.^{21,22} Similarly, aminobenzimidazole 1 (AB-1) (**III**) has been demonstrated as a potent inhibitor of GyrB, while exhibiting a minimum inhibitory concentration (MIC) of $1 \mu\text{g L}^{-1}$ against the H37Ra *Mtb* strain.²³ There are also some benzimidazole-derived hybrid molecules that have been developed as potent drugs and are being effectively used for the treatment of different diseases. These include: albendazole (**IV**), having antimicrobial activity;^{24,25} omeprazole (**V**), as an antiulcer drug;^{26,27} and bendamustine (**VI**), regarded as one of the effective anticancer drugs (Fig. 1).^{28,29}

Similarly to benzimidazole hybrids, 2-aminothiazoles are also a familiar group of heterocyclic compounds that exhibit a broad range of biological activities, and their advantages as medicines have been well established.^{30,31} For example, the thiazole nucleus is an integral component (main core) of all available penicillins, which have transmuted the therapy of bacterial diseases.³² In fact, aminothiazole derivatives have attracted the medicinal community with regards to the synthesis of combinatorial heterocyclic compound series possessing various biological or therapeutic activities, particularly against *Mtb*.^{16,33} The synthetic dibenzofuran-, carbazole- and dibenzothiophene-derived thiazolamine derivatives **VII–IX** have been shown to possess potent antitubercular activity against the H37Ra *Mtb* strain, with an MIC range of $0.78\text{--}1.56 \mu\text{g mL}^{-1}$, respectively.^{33,34} Similarly,

the naturally occurring antibiotic thiolactomycin (TLM) analogs **X** and **XI**, which primarily act by inhibiting the FAS-II β -ketoacyl-ATP synthase condensing enzyme, have been demonstrated to halt mycolic acid biosynthesis and then subsequently induce *Mtb* cell death.^{35–38} Moreover, nitazoxanide (NTZ, **XII**) and its active metabolite tizoxanide (TIZ, **XIII**) are other examples of aminothiazole-based drugs that have been manifested to inhibit replicative and non-replicative strains of *Mtb*.^{39,40} The good remedial properties of these related drugs have inspired medicinal chemists to synthesize a number of novel clinical agents to cure various types of diseases.^{16,18,41}

Indeed, the molecular hybridization approach has emerged as a useful structural modification approach for the design of ligands with new molecular architectures.⁴² In this work, in an effort to identify new antitubercular agents, we report the synthesis and biological evaluation of a novel series of benzimidazolallylidenehydrazinylmethylthiazole derivatives that we designed through the hybridization of antitubercular pharmacophores like benzimidazole, aminothiazole and unsaturated aromatic substitution units. The designed scaffold (Fig. 3) originated from the structures of the antitubercular agents **VII**, **VIII** and **IX** (Fig. 2). The newly designed hybridized molecule comprises three parts: (1) a 2-aminothiazole ring, as an antitubercular active core unit; (2) benzimidazole, as an antitubercular pharmacophoric backbone unit; and (3) substituted aromatic unsaturated groups appended to the benzimidazole and aminothiazole units, for enhancing the pharmacophoric impact and to induce a lipophilic nature. The presence of an allyl group may cause a decrease in lipophilicity and, as a result, diminish the compound's ability to penetrate the thick lipophilic cell wall of *Mtb*.⁴³ Therefore, variations in the designed hybrid molecule were accomplished with the choice of substituted aromatic unsaturated groups, aiming for greater lipophilicity, which may assist in potentiating the anti-TB activity of the designed series. The target compounds **8a–o** were synthesized

Fig. 1 Synthetic bioactive analogs of benzimidazole.

Fig. 2 Synthetic bioactive analogs of aminothiazole.

through a multi-step synthetic sequence utilizing simple condensation reactions. The *in vitro* antimycobacterial screening of the designed library led to the identification of an important hit compound, which may be pursued for future lead optimization studies.

2. Results and discussion

2.1. Chemistry

Initially, as depicted in Scheme 1, we started with the synthesis of 1-(1*H*-benzo[*d*]imidazol-2-yl)ethanone (4). For this purpose, we conducted the cyclisation of benzene-1,2-diamine (1) in the presence of lactic acid (2) and 4*N* hydrochloric acid to form 1-(1*H*-benzo[*d*]imidazol-2-yl)ethanol (3), and then oxidized compound 3 with chromium trioxide in the presence of acetic acid to form the required 1-(1*H*-benzo[*d*]imidazol-2-yl)ethanone (4) in 62% yield. All compounds were fully characterized *via* NMR and ESI-mass spectral analysis.⁴⁴

Compound 4 was treated with the various substituted aromatic aldehydes 5a–o and sodium hydroxide under aldol condensation conditions to form the substituted (*E*)-1-(1*H*-

benzo[*d*]imidazol-2-yl)-3-phenylprop-2-en-1-one derivatives 6a–o. Compounds 6a–o were then condensed with thiosemicarbazide in the presence of concentrated hydrochloric acid and ethanol under reflux to form the benzimidazolallylidenehydrazinecarbothioamide derivatives 7a–o in good yields. Finally, the substituted benzimidazolallylidenehydrazinecarbothioamides 7a–o were treated with chloroacetone in methanol under reflux to form the new desired benzimidazolallylidenehydrazinylmethylthiazole derivatives 8a–o in good yields (Table 1). All synthesized compounds were fully characterized using ¹H and ¹³C NMR, IR and mass (ESI-MS & HR-MS) spectral data.^{44,45} The log *P* and clog *P* values required to assess the lipophilic character of the new analogs were calculated using the ChemBiodraw 12.0 program (Table 1).

2.2. Pharmacology

2.2.1. Antimycobacterial activities. All the newly synthesized benzimidazolallylidenehydrazinylmethylthiazole derivatives 8a–o and precursor compounds 7a–o were screened for *in vitro* antimycobacterial activity against the selectable marker-free auto-luminescent *Mtb* H₃₇Ra strain,^{46,47} while using the first-line anti-TB drug rifampicin as a positive control. The MIC (minimum concentration required to inhibit 90% of bacterial growth) values ($\mu\text{g mL}^{-1}$) for 7a–o and 8a–o along with rifampicin (for comparison) are listed in Table 1. All thirty compounds (fifteen final derivatives and fifteen precursor derivatives) showed a certain level of *in vitro* antimycobacterial activity against *Mtb*, with MIC values ranging from 2.5–100 $\mu\text{g mL}^{-1}$. The majority of the compounds exhibited moderately potent MIC values (*i.e.*, less than or equal to 10 $\mu\text{g mL}^{-1}$). In particular, compounds 7e and 8g were found to be the most active ones, both possessing MIC values of 2.5 $\mu\text{g mL}^{-1}$. However, when compared to the control drug rifampicin (MIC: 0.25 $\mu\text{g mL}^{-1}$), all new compounds were found to be less potent.

Fig. 3 The design strategy for the synthesis of benzimidazolallylidenehydrazinylmethylthiazole derivatives.

Scheme 1 The synthesis of novel benzimidazolallylidenehydrazinecarbothioamide (**7a-o**) and benzimidazolallylidenehydrazinylmethylthiazole (**8a-o**) derivatives. Reagents and conditions: (a) 4N HCl, reflux, 10 h; (b) CrO₃, AcOH, 90–105 °C, 30 min; (c) NaOH, EtOH, 0 °C – RT, 8 h; (d) NH₂SNHNNH₂, conc. HCl, EtOH, reflux, 5 h; and (e) ClCH₂COCH₃, MeOH, reflux, 3 h.

2.2.2. *In vitro* cytotoxicity. The *in vitro* cytotoxicity values of all the most suitable antitubercular active analogues were assessed using Cell Counting Kit-8 (CCK-8) against Madin-Darby Canine Kidney (MDCK) cells at different concentrations. The inhibitory cell concentrations are reported in Fig. 4. The IC₅₀ values of the most promising antitubercular compounds **7a**, **7b**, **7d**, **7e**, **7f**, **7j**, **7k**, **7l**, **7m**, **7n**, **8a**, **8e** and **8g** were found to be 3.4, 3.7, 0.34, 1.6, 2.6, 0.91, 1.6, 1.5, 1.6, 3.9, 2.9, 1.9 and >30 µg mL⁻¹, respectively. These results indicate that the potent analog **8g** that we identified is less toxic, and is suitable for further drug development processes.

2.3. Structure–activity relationship (SAR)

To begin the SAR studies, we first investigated the antitubercular potentials of the benzimidazolallylidenehydrazinecarbothioamide derivatives (**7a-o**) through modifications to the olefinic system. We found that *p*-bromo phenyl (MIC: 4 µg mL⁻¹; **7f**) and *p*-fluoro phenyl (MIC: 2.5 µg mL⁻¹; **7e**) substitutions were a little more potent compared to simple phenyl (**7a**; MIC: 5 µg mL⁻¹) and *p*-tolyl (**7b**; MIC: 5 µg mL⁻¹) substitutions. We also probed the Mtb inhibition activ-

ities by introducing di-substituted phenyl systems into the benzimidazolallylidenehydrazinecarbothioamide. In this case, di-halo substituted phenyl rings with 4-chloro-3-fluoro (**7l**) and 3,5-di-fluoro phenyl (**7m**) substitutions (both having MIC values of 5 µg mL⁻¹) were slightly less potent than the phenyl ring with 4-fluoro-3-methyl substitution (**7k**; MIC: 4 µg mL⁻¹). This indicated that mono- or di-halo substituted phenyl derivatives exhibit more potent Mtb inhibition with 4-fluoro phenyl substitutions.

Next, we modified the phenyl ring with electron donating groups, for example with methoxy substitution at different positions. In this case, 2,3-dimethoxy phenyl (**7n**; MIC: 5 µg mL⁻¹) demonstrated more potent activity than *p*-methoxy phenyl (**7c**; MIC: 10 µg mL⁻¹) substitution; whereas, 2,4,6-trimethoxy phenyl substitution was found to be worse in activity (**7o**; MIC: > 50 µg mL⁻¹). From the perspective of electron withdrawing substitutions on the phenyl ring, we found that *p*-trifluoromethoxy substitution (**7d**; MIC: 5 µg mL⁻¹) resulted in better activity compared to *p*-methoxy substitution on the phenyl ring (**7c**; MIC: 10 µg mL⁻¹). We further altered the *p*-trifluoromethoxy substitution with an electron donating group, for example with *p*-*N,N*-diethyl

Table 1 Antimycobacterial activity of benzimidazolallylidenehydrazinecarbothioamide (7a–o), and benzimidazolallylidenehydrazinylmethylthiazole (8a–o) derivatives

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>7a-o</p> </div> <div style="text-align: center;"> <p>8a-o</p> </div> </div>						
Entry	R	Product ^a	Yield ^b (%)	Log <i>p</i> ^c	clog <i>P</i> ^c	MIC ₉₀ (μg mL ⁻¹)
1.	5a	7a	65	2.93	4.93	5
		8a	60	5.07	4.99	4
2.	5b	7b	62	3.42	5.43	5
		8b	60	5.56	5.49	10
3.	5c	7c	55	2.80	4.85	10
		8c	53	4.95	4.91	10
4.	5d	7d	56	4.46	5.96	5
		8d	54	6.60	6.02	10
5.	5e	7e	61	3.09	5.08	2.5
		8e	58	5.23	5.14	5
6.	5f	7f	54	3.76	5.80	4
		8f	55	5.90	5.86	10
7.	5g	7g	50	3.89	6.16	10
		8g	52	6.03	6.22	2.5
8.	5h	7h	58	2.49	4.23	10
		8h	55	4.64	4.29	10
9.	5i	7i	60	1.47	3.54	100
		8i	52	3.62	3.60	100
10.	5j	7j	53	2.47	4.92	5
		8j	61	4.61	4.98	10
11.	5k	7k	63	3.58	5.58	4
		8k	65	5.72	5.64	10
12.	5l	7l	52	3.65	5.79	5
		8l	60	5.79	5.85	10
13.	5m	7m	55	3.25	5.22	5
		8m	58	5.39	5.28	10
14.	5n	7n	62	2.68	4.59	5
		8n	61	4.82	4.65	10
15.	5o	7o	58	2.55	4.93	>50
		8o	55	4.69	4.99	>50
16.	Rifampicin	—	—	—	—	0.25

^a Benzimidazolyl substituted arylpropenone (1.0 mmol), thiosemicarbazide (1.1 mmol) and benzimidazolallylidenehydrazinecarbothioamide (1.0 mmol), and chloroacetone (1.1 mmol). ^b Isolated yield. ^c Calculated using Chembiodraw 12.0.

substitution on the phenyl ring (7g). However, the potency was slightly decreased (MIC: 10 μg mL⁻¹). Based on these findings, we concluded that strong electronegative groups at the *p*-position of the phenyl ring exhibit a good activity profile (*i.e.* potency) compared to *p*-electropositive groups.

We also investigated the activity profiles of the newly synthesized compounds by altering the phenyl substitutions with heterocyclic groups on the olefinic chain. We observed that the potency of indole substitution (7j; MIC: 5 μg mL⁻¹) was better in comparison to 2-chloropyridine (7h; MIC: 10 μg mL⁻¹) substitution and was far better than pyrrole (7i; MIC: 100 μg mL⁻¹) substitution.

Finally, we studied the activity profiles of aminothiazole derivatives (8a–o) derived from benzimidazolallylidene-

hydrazinecarbothioamides (7a–o), following the same SAR pattern as for 7a–o. Here, the Mtb activity decreased from all kinds of substitutions, except the electron donating group (*p*-*N,N*-diethyl phenyl) on 7g, which resulted in a 4-fold increase in potency when it was converted to its corresponding aminothiazole 8g (MIC: 2.5 μg mL⁻¹). Moreover, no change in activity was observed when 7c, 7i, and 7o were converted to their corresponding 8c (MIC: 10 μg mL⁻¹), 8i (MIC: 100 μg mL⁻¹), and 8o (MIC > 50 μg mL⁻¹) aminothiazole derivatives.

3. Conclusions

We have designed and synthesized a series of novel benzimidazolallylidenehydrazinecarbothioamide derivatives

Fig. 4 IC₅₀ values of the novel benzimidazolylallylidenehydrazinecarbothioamides 7a–o and benzimidazolylallylidenehydrazinylmethylthiazoles 8a–o against MDCK cells.

7a–o and benzimidazolylallylidenehydrazinylmethylthiazole derivatives 8a–o *via* multi-step condensation reactions. All new derivatives were fully analyzed *via* NMR and mass spectral analysis. We also explored the SAR of the synthesized compounds in relation to antitubercular activity against the H₃₇Ra Mtb strain. From *in vitro* antimycobacterial screening assays, we have identified compounds 7e and 8g (MIC: 2.5 µg mL⁻¹) as the most active antitubercular agents among the synthesized series. Moreover, compound 8g exhibited a lower cytotoxicity profile. We conceive that compound 8g may be regarded as a drug-like hit compound and may serve as a chemical probe of interest for further SAR and lead optimization studies aimed at developing novel and effective antitubercular agents.

4. Experimental section

4.1. Chemistry

4.1.1. General. All reagents were purchased from Acros, Fluka, Sigma, Aldrich or Merck and used without further purification. All solvents were purchased from commercial suppliers. ¹H NMR and ¹³C NMR spectra were recorded on 400 MHz or/and 500 MHz Bruker FT-NMR spectrometers. All chemical shifts are given as δ values (ppm) with reference to tetramethylsilane (TMS = 0) as an internal standard. The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; q, quartet. The coupling constants, *J*, are reported in Hertz (Hz). Analytical HPLC was performed with an Agilent 1200 Series system using a Waters Acquity UPLC BEH 250 × 4.6 mm, C18, 5 µm column (solvent: MeOH – water + 0.1% NH₃H₂O; gradient: 1 ml min⁻¹; *T* = 25 °C) and UV detection at λ = 210 nm. High resolution mass (MS) analysis was conducted using an LC/MSD TOF spectrometer system with electrospray ionization (ESI). Reactions were monitored *via* thin-layer chromatography (TLC) carried out on commercial silica gel plates (GF254) under UV light. Column chromatography was performed on silica gel 60 (200–300 mesh).

4.1.2. General procedure for the synthesis of 1-(1*H*-benzo[*d*]imidazol-2-yl)ethanol (3). A mixture of benzene-1,2-diamine (5 g, 46.29 mmol) and lactic acid (50.92 mmol) in 4*N* HCl (30 mL) was refluxed for 10 h; after the disappearance of the reactant (monitored by TLC), the reaction mixture was cooled to room temperature and poured into ice cold water. The reaction mixture was neutralized with ammonia. The desired product was formed as a white solid, filtered and recrystallized with ethanol to form our desired pure compound with 70% yield.

4.1.3. General procedure for the synthesis of 1-(1*H*-benzo[*d*]imidazol-2-yl)ethanone (4). 1-(1*H*-benzo[*d*]imidazol-2-yl)ethanol (3) (1 g, 6.17 mmol) was dissolved in 10 mL of acetic acid and heated to 90 °C. After 10 minutes, chromium trioxide (4.62 mmol) in 2 mL of water was slowly added dropwise to the reaction mixture and it was stirred at 105 °C for 30 min. After completion of the reaction (monitored by TLC), the crude residue was cooled to room temperature and poured into ice cold water. A brick-red colored solid compound was formed and filtered. The desired solid compound was recrystallized with ethanol and dried on a rotary evaporator to obtain the pure compound in 62% yield.

4.1.4. General procedure for the synthesis of substituted benzoimidazolyl propenone (6a–o). To a solution of 1-(1*H*-benzo[*d*]imidazol-2-yl)ethanone (4) (0.1 mmol) and substituted aromatic aldehydes (5a–o) (0.1 mmol) in ethanol at 0 °C, ethanolic sodium hydroxide (0.2 mmol) was added slowly and the mixture was stirred at room temperature for 8 h. A yellow solid precipitate was formed, and the solvent was evaporated on a rotary evaporator. The crude residue was washed with chloroform and water. The organic layer was separated from the aqueous layer, and the compound was purified *via* silica gel column chromatography. All the purified benzoimidazolyl propenones (6a–o) were formed in good yields.

4.1.4.1. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-phenylprop-2-en-1-one (6a). Yield: 65%; ¹H NMR (400 MHz, CDCl₃): δ 10.83 (br s, 1H), 8.18–8.07 (m, 2H), 7.96 (d, *J* = 14.2 Hz, 1H), 7.76 (t, *J* =

5.6 Hz, 2H), 7.59 (d, J = 14.1 Hz, 1H), 7.46–7.36 (m, 5H); MS (ESI): m/z 271 ($M + Na$)⁺.

4.1.4.2. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(*p*-tolyl)prop-2-en-1-one (**6b**). Yield: 68%; ¹H NMR (500 MHz, CDCl₃): δ 10.38 (br s, 1H), 8.08 (s, 3H), 7.96 (d, J = 13.9 Hz, 1H), 7.66 (d, J = 8.1 Hz, 2H), 7.56 (d, J = 14.3 Hz, 1H), 7.44 (t, J = 8.1 Hz, 1H), 7.38 (t, J = 8.5 Hz, 1H), 7.24 (s, 1H), 2.41 (s, 3H); MS (ESI): m/z 285 ($M + Na$)⁺.

4.1.4.3. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (**6c**). Yield: 67%; ¹H NMR (400 MHz, CDCl₃): δ 10.52 (br s, 1H), 8.04 (d, J = 8.1 Hz, 2H), 7.95 (d, J = 13.8 Hz, 1H), 7.73 (d, J = 8.5 Hz, 2H), 7.58 (d, J = 14.0 Hz, 1H), 7.46–7.35 (m, 2H), 6.96 (d, J = 8.5 Hz, 2H), 3.87 (s, 3H); MS (ESI): m/z 301 ($M + Na$)⁺.

4.1.4.4. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(trifluoromethoxy)phenyl)prop-2-en-1-one (**6d**). Yield: 60%; ¹H NMR (400 MHz, CDCl₃): δ 10.57 (br s, 1H), 8.08 (q, J = 8.1 Hz, 7.8 Hz, 2H), 7.96 (d, J = 13.5 Hz, 1H), 7.78 (d, J = 8.5 Hz, 2H), 7.59 (d, J = 13.8 Hz, 1H), 7.45 (t, J = 7.0 Hz, 1H), 7.39 (t, J = 7.0 Hz, 1H), 7.28 (d, J = 8.0 Hz, 2H); MS (ESI): m/z 333 ($M + H$).

4.1.4.5. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-fluorophenyl)prop-2-en-1-one (**6e**). Yield: 58%; ¹H NMR (400 MHz, CDCl₃): δ 10.55 (br s, 1H), 8.05 (s, 2H), 7.95 (d, J = 13.9 Hz, 1H), 7.76 (q, J = 5.5 Hz, 2H), 7.58 (d, J = 13.9 Hz, 1H), 7.48–7.36 (m, 2H), 7.13 (t, J = 8.5 Hz, 1H); MS (ESI): m/z 289 ($M + Na$)⁺.

4.1.4.6. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-bromophenyl)prop-2-en-1-one (**6f**). Yield: 62%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.19 (s, 1H), 8.14 (d, J = 14.1 Hz, 1H), 7.93 (d, J = 15.2 Hz, 1H), 7.71 (s, 1H), 7.68–7.56 (m, 5H), 7.35 (s, 2H); MS (ESI): m/z 327 ($M + H$)⁺.

4.1.4.7. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(diethylamino)phenyl)prop-2-en-1-one (**6g**). Yield: 65%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.21 (br s, 1H), 7.96–7.76 (m, 3H), 7.63 (d, J = 14.2 Hz, 2H), 7.57 (d, J = 14.8 Hz, 1H), 7.41–7.23 (m, 2H), 6.72 (d, J = 8.5 Hz, 2H), 3.46 (q, J = 7.07 Hz, 6.80 Hz, 4H), 1.19 (t, J = 6.8 Hz, 6H); MS (ESI): m/z 320 ($M + H$)⁺.

4.1.4.8. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(2-chloropyridin-3-yl)prop-2-en-1-one (**6h**). Yield: 55%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.64 (br s, 1H), 8.63 (d, J = 8.5 Hz, 1H), 8.55 (d, J = 14.2 Hz, 1H), 8.22 (q, J = 6.5 Hz, 7.1 Hz, 2H), 7.83 (s, 2H), 7.60 (q, J = 15.5 Hz, 1H), 7.43 (s, 2H); MS (ESI): m/z 282 ($M - H$)⁺.

4.1.4.9. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(1*H*-pyrrol-2-yl)prop-2-en-1-one (**6i**). Yield: 58%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.09 (br s, 1H), 11.82 (s, 1H), 7.83 (s, 2H), 7.77 (d, J = 13.8 Hz, 1H), 7.58 (d, J = 14.2 Hz, 1H), 7.39–7.25 (m, 2H), 7.05 (s, 1H), 6.70 (s, 1H), 6.25 (s, 1H); MS (ESI): m/z 260 ($M + Na$).

4.1.4.10. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(1*H*-indol-4-yl)prop-2-en-1-one (**6j**). Yield: 60%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.47 (br s, 1H), 11.55 (br s, 1H), 8.29 (s, 2H), 7.91 (d, J = 15.2 Hz, 1H), 7.65–7.56 (m, 4H), 7.44–7.33 (m, 2H), 7.23 (d, J = 14.8 Hz, 1H), 6.91 (s, 1H); MS (ESI): m/z 288 ($M + H$).

4.1.4.11. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-fluoro-3-methyl phenyl)prop-2-en-1-one (**6k**). Yield: 62%; ¹H NMR (500 MHz, DMSO-*d*₆): δ 8.12 (d, J = 15.4 Hz, 1H), 7.92 (t, J = 8.5 Hz, 2H), 7.75 (s, 3H), 7.38 (s, 2H), 7.28 (d, J = 14.5 Hz, 1H), 2.34 (s, 2H); MS (ESI): m/z 281 ($M + H$)⁺.

4.1.4.12. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-chloro-3-fluorophenyl)prop-2-en-1-one (**6l**). Yield: 59%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.55 (s, 1H), 8.19 (d, J = 16.1 Hz, 1H), 8.05–7.95 (m, 1H), 7.90 (d, J = 15.6 Hz, 1 Hz), 7.80 (t, J = 4.0 Hz, 1H), 7.76 (d, J = 15.2 Hz, 1H), 7.71 (d, J = 8.2 Hz, 1H), 7.61 (d, J = 4.1 Hz, 1H), 7.44 (d, J = 8.1 Hz, 1H), 7.38 (d, J = 8.2 Hz, 1H); MS (ESI): m/z 301 ($M + H$)⁺.

4.1.4.13. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(3,5-difluorophenyl)prop-2-en-1-one (**6m**). Yield: 57%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.79 (br s, 1H), 9.01 (d, J = 15.9 Hz, 1H), 8.65 (d, J = 7.8 Hz, 1H), 7.83 (d, J = 15.9 Hz, 1H), 7.76 (d, J = 15.6 Hz, 1H), 7.55 (d, J = 7.5 Hz, 1H), 7.49 (t, J = 8.0 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.27 (m, 2H); MS (ESI): m/z 285 ($M + H$)⁺.

4.1.4.14. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one (**6n**). Yield: 62%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.48 (s, 1H), 8.15 (q, J = 8.4 Hz, 8.1 Hz, 2H), 7.87 (s, 1H), 7.60 (s, 1H), 7.50 (d, J = 14.5 Hz, 1H), 7.38 (t, J = 14.8 Hz, 2H), 7.24–7.18 (m, 2H), 3.85 (s, 6H); MS (ESI): m/z 309 ($M + H$)⁺.

4.1.4.15. (*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**6o**). Yield: 65%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.22 (br s, 1H), 8.27 (s, 2H), 7.84 (d, J = 14.2 Hz, 1H), 7.56 (d, J = 14.8 Hz, 1H), 7.42–7.25 (m, 2H), 6.35 (s, 2H), 3.95 (s, 6H), 3.88 (s, 3H); MS (ESI): m/z 339 ($M + H$)⁺.

4.1.5. General procedure for the synthesis of the benzimidazolylallylidenehydrazinecarbothioamides (**7a–o**). To a mixture of substituted benzoimidazolylpropenones (**6a–o**) (0.1 mmol) and thiosemicarbazide (0.12 mmol) in ethanol, a catalytic amount of concentrated HCl was added, and this was refluxed for 8 h. After the completion of the reaction (monitored by TLC), the solvent was evaporated on a rotary evaporator. The solid compound was washed with water and EtOAc. The organic layer was treated with Na₂SO₄ and the compound was purified with silica gel column chromatography. The desired light yellow colored pure compounds were obtained in good yields.

4.1.5.1. (*Z*)-2-((*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-phenylallylidene)hydrazinecarbothioamide (**7a**). Yield: 65%; m.p.: 143–144 °C; ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.76 (br s, 1H), 11.53 (br s, 1H), 8.67 (d, J = 8.2 Hz, 2H), 8.49 (d, J = 15.1 Hz, 1H), 7.87–7.79 (m, 3H), 7.75 (t, J = 7.8 Hz, 1H), 7.53 (d, J = 15.5 Hz, 1H), 7.49 (m, 3H), 7.30 (t, J = 7.5 Hz, 1H), 7.21 (t, J = 7.5 Hz, 1H); ¹³C NMR (125 MHz, DMSO-*d*₆): δ 180.0, 150.2, 142.0, 137.4, 130.1129.5, 128.9, 128.1, 124.4, 122.5, 116.3, 112.0; IR (KBr): 3250, 2925, 1572, 1452, 1352, 1150, 952, 757 cm⁻¹; MS (ESI): m/z 322 ($M + H$)⁺.

4.1.5.2. (*Z*)-2-((*E*)-1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(*p*-tolyl)allylidene)hydrazinecarbothioamide (**7b**). Yield: 62%; m.p.: 132–134 °C; ¹H NMR (500 MHz, CDCl₃ + DMSO-*d*₆): δ 12.68

(s, 1H), 11.44 (s, 1H), 8.61 (br s, 2H), 8.49 (d, $J = 15.5$ Hz, 1H), 7.76–7.67 (m, 2H), 7.66–7.63 (m, 1H), 7.50 (d, $J = 7.1$ Hz, 1H), 7.35 (d, $J = 14.8$ Hz, 1H), 7.34–7.21 (m, 3H), 6.88 (d, $J = 7.5$ Hz, 1H), 2.37 (s, 3H); ^{13}C NMR (125 MHz, $\text{CDCl}_3 + \text{DMSO-d}_6$): δ 179.0, 149.2, 141.1, 139.1, 133.7, 129.3, 129.2, 128.3, 128.0, 127.27, 127.24, 114.3, 21.0; IR (KBr): 3252, 2923, 1575, 1435, 1345, 1085, 735 cm^{-1} ; MS (ESI): m/z 336 ($\text{M} + \text{H}$) $^+$.

4.1.5.3. (Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(4-methoxyphenyl)allylidene)hydrazinecarbothioamide (7c). Yield: 55%; m.p: 116–118 $^{\circ}\text{C}$; ^1H NMR (400 MHz, DMSO-d_6): δ 12.66 (s, 1H), 11.39 (s, 1H), 8.59 (br s, 2H), 8.48 (d, $J = 8.5$ Hz, 2H), 7.79 (d, $J = 14.2$ Hz, 1H), 7.69–7.61 (m, 2H), 7.30–7.20 (m, 3H), 6.98 (d, $J = 14.6$ Hz, 1H), 6.93 (d, $J = 8.5$ Hz, 1H), 6.62 (d, $J = 8.5$ Hz, 1H), 3.83 (s, 3H); IR (KBr): 3252, 2856, 1575, 1345, 1183, 955, 735 cm^{-1} ; MS (ESI): m/z 352 ($\text{M} + \text{H}$) $^+$.

4.1.5.4. (Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(4-trifluoromethoxyphenyl)allylidene)hydrazinecarbothioamide (7d). Yield: 56%; m.p: 128–130 $^{\circ}\text{C}$; ^1H NMR (400 MHz, DMSO-d_6): δ 12.77 (br s, 1H), 11.56 (br s, 1H), 8.69 (d, $J = 21.7$ Hz, 2H), 8.50 (d, $J = 14.9$ Hz, 1H), 7.96 (d, $J = 8.8$ Hz, 2H), 7.78 (d, $J = 15.2$ Hz, 1H), 7.44 (d, $J = 8.0$ Hz, 2H), 7.34–7.17 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 180.1, 150.1, 149.6, 140.1, 136.8, 136.7, 136.3, 130.7, 129.8, 122.2, 121.9, 117.5; IR (KBr): 3250, 2920, 1565, 1475, 1355, 1152, 955 cm^{-1} ; MS (ESI): m/z 406 ($\text{M} + \text{H}$) $^+$.

4.1.5.5. (Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(4-fluorophenyl)allylidene)hydrazinecarbothioamide (7e). ^1H NMR (500 MHz, $\text{CDCl}_3 + \text{DMSO-d}_6$): Yield: 61%; HPLC purity: 95%; m.p: 135–137 $^{\circ}\text{C}$; δ 11.78 (s, 1H), 8.87 (d, $J = 15.1$ Hz, 2H), 7.88 (q, $J = 8.5$ Hz, 7.8 Hz, 2H), 7.82 (q, $J = 7.2$ Hz, 6.9 Hz, 2H), 7.70 (d, $J = 15.9$ Hz, 1H), 7.58–7.50 (m, 3H), 7.26 (t, $J = 8.5$ Hz, 2H); ^{13}C NMR (75 MHz, $\text{CDCl}_3 + \text{DMSO-d}_6$): δ 179.3, 146.6, 143.8, 139.0, 138.4, 130.2, 130.1, 129.47, 129.40, 127.5, 125.5, 115.7, 115.5, 115.3, 115.1, 114.6, 114.0; IR (KBr): 3320, 2865, 1575, 1455, 1350, 1175, 952, 735 cm^{-1} ; MS (ESI): m/z 340 ($\text{M} + \text{H}$) $^+$.

4.1.5.6. (Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(4-bromophenyl)allylidene)hydrazinecarbothioamide (7f). ^1H NMR (400 MHz, DMSO-d_6): yield: 54%; m.p: 155–157 $^{\circ}\text{C}$; δ 12.77 (s, 1H), 11.57 (s, 1H), 8.68 (d, $J = 8.3$ Hz, 2H), 8.47 (d, $J = 15.9$ Hz, 2H), 7.84–7.71 (m, 3H), 7.65 (d, $J = 15.3$ Hz, 1H), 7.53 (d, $J = 14.9$ Hz, 1H), 7.36–7.16 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 179.0, 149.1, 139.5, 135.7, 135.3, 131.5, 129.8, 122.3, 116.1; IR (KBr): 3265, 2920, 1565, 1435, 1352, 935, 752 cm^{-1} ; MS (ESI): m/z 402 ($\text{M} + 2$) $^+$.

4.1.5.7. (Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(4-(diethylamino)phenyl)allylidene)hydrazinecarbothioamide (7g). Yield: 50%; m.p: 152–154 $^{\circ}\text{C}$; ^1H NMR (400 MHz, DMSO-d_6): δ 12.90 (br s, 1H), 8.25 (br s, 1H), 7.69 (d, $J = 15.6$ Hz, 1H), 7.57 (d, $J = 8.0$ Hz, 2H), 7.31 (t, $J = 7.8$ Hz, 1H), 7.23 (t, $J = 7.8$ Hz, 1H), 6.96 (d, $J = 8.3$ Hz, 2H), 6.57 (d, $J = 14.2$ Hz, 2H), 5.82 (dd, $J = 8.3$ Hz & 7.5 Hz, 1H), 3.28 (q, $J = 7.0$ Hz, 4H), 1.05 (t, $J = 6.8$ Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 176.5, 147.2, 146.4, 144.8, 134.3, 128.3, 126.5, 124.1, 122.0, 119.4, 111.5, 111.1, 43.4, 12.2; IR (KBr): 3256, 1626, 1435, 1352, 1225, 1055, 785 cm^{-1} ; MS (ESI): m/z 393 ($\text{M} + \text{H}$) $^+$.

4.1.5.8. (Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(2-chloropyridin-3-yl)allylidene)hydrazinecarbothioamide (7h). Yield: 58%; m.p: 82–84 $^{\circ}\text{C}$; ^1H NMR (400 MHz, DMSO-d_6): δ 12.84 (s, 1H), 11.66 (s, 1H), 8.94 (d, $J = 15.6$ Hz, 1H), 8.77 (d, $J = 17.8$ Hz, 2H), 8.68 (d, $J = 7.8$ Hz, 1H), 8.46 (s, 1H), 7.88 (d, $J = 15.6$ Hz, 1H), 7.75 (d, $J = 8.0$ Hz, 1H), 7.59 (q, $J = 7.04$ Hz, 2H), 7.34 (t, $J = 7.8$ Hz, 1H), 7.25 (t, $J = 7.8$ Hz, 1H); IR (KBr): 3255, 1565, 1455, 1235, 1055, 852, 785 cm^{-1} ; MS (ESI): m/z 357 ($\text{M} + \text{H}$) $^+$.

4.1.5.9. (Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(1H-pyrrol-2-yl)allylidene)hydrazinecarbothioamide (7i). Yield: 60%; m.p: 110–112 $^{\circ}\text{C}$; ^1H NMR (400 MHz, DMSO-d_6): δ 12.86 (br s, 1H), 11.78 (br s, 1H), 7.72 (d, $J = 14.5$ Hz, 1H), 7.58 (d, $J = 14.2$ Hz, 2H), 7.33 (t, $J = 8.0$ Hz, 1H), 7.24 (t, $J = 8.0$ Hz, 1H), 6.77–6.57 (m, 2H), 6.02 (dd, $J = 8.0$ Hz, 7.5 Hz, 1H), 5.94–5.86 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 176.5, 147.9, 144.7, 134.3, 130.7, 124.1, 122.0, 119.4, 117.6, 111.5, 106.8, 105.3; IR (KBr): 3275, 2865, 1652, 1535, 1425, 1250, 1055, 783 cm^{-1} ; MS (ESI): m/z 309 ($\text{M} - \text{H}$) $^+$.

4.1.5.10. (Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(1H-indol-4-yl)allylidene)hydrazinecarbothioamide (7j). Yield: 53%; m.p: 147–149 $^{\circ}\text{C}$; ^1H NMR (400 MHz, DMSO-d_6): δ 12.78 (br s, 1H), 11.50 (br s, 1H), 11.30 (br s, 1H), 9.02 (d, $J = 8.1$ Hz, 1H), 8.64 (d, $J = 8.2$ Hz, 2H), 7.83 (d, $J = 15.9$ Hz, 1H), 7.77 (d, $J = 15.6$ Hz, 1H), 7.57–7.44 (m, 2H), 7.35–7.15 (m, 3H), 6.74 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 180.0, 150.5, 140.7, 137.8, 137.2, 128.7, 128.4, 127.2, 124.4, 122.4, 121.9, 120.5, 118.4, 115.5, 113.5, 100.4; IR (KBr): 3257, 1621, 1456, 1413, 1378, 1256, 1145, 817, 729, 661 cm^{-1} ; MS (ESI): m/z 361 ($\text{M} + \text{H}$) $^+$.

4.1.5.11. (Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(4-fluoro-3-methylphenyl)allylidene)hydrazinecarbothioamide (7k). Yield: 63%; m.p: 140–142 $^{\circ}\text{C}$; ^1H NMR (500 MHz, DMSO-d_6): δ 12.79 (br s, 1H), 11.48 (s, 1H), 8.68 (d, $J = 8.0$ Hz, 1H), 8.45 (d, $J = 15.6$ Hz, 1H), 7.73 (t, $J = 7.8$ Hz, 3H), 7.53 (d, $J = 15.2$ Hz, 1H), 7.33 (m, 3H), 2.30 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 180.0, 163.1, 161.1, 150.1, 140.9, 133.6, 132.2, 128.27, 128.21, 125.5, 125.3, 116.0, 115.9, 14.9; IR (KBr): 3252, 2853, 1565, 1455, 1055, 745 cm^{-1} ; MS (ESI): m/z 354 ($\text{M} + \text{H}$) $^+$.

4.1.5.12. (Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(4-chloro-3-fluorophenyl)allylidene)hydrazinecarbothioamide (7l). Yield: 52%; m.p: 122–124 $^{\circ}\text{C}$; ^1H NMR (400 MHz, DMSO-d_6): δ 12.78 (br s, 1H), 11.54 (s, 1H), 8.02 (d, $J = 8.3$ Hz, 1H), 7.83 (t, $J = 8.0$ Hz, 1H), 7.78 (d, $J = 14.5$ Hz, 1H), 7.73 (d, $J = 7.5$ Hz, 1H), 7.69 (d, $J = 15.2$ Hz, 1H), 7.63 (t, $J = 8.8$ Hz, 2H), 7.29 (t, $J = 7.3$ Hz, 1H), 7.22 (t, $J = 7.4$ Hz, 1H); IR (KBr): 3270, 2851, 1592, 1488, 1456, 1370, 1357, 1172, 964, 766 cm^{-1} ; MS (ESI): m/z 374 ($\text{M} + \text{H}$) $^+$.

4.1.5.13. (Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(3,5-difluorophenyl)allylidene)hydrazinecarbothioamide (7m). Yield: 55%; m.p: 142–144 $^{\circ}\text{C}$; ^1H NMR (400 MHz, DMSO-d_6): δ 12.81 (br s, 1H), 11.58 (br s, 1H), 8.80 (s, 1H), 7.72 (s, 1H), 8.46 (d, $J = 8.2$ Hz, 1H), 7.86 (s, 1H), 7.75 (d, $J = 14.3$ Hz, 1H), 7.63 (t, $J = 8.3$ Hz, 2H), 7.55 (d, $J = 7.8$ Hz, 1H), 7.43 (d, $J = 14.8$ Hz, 1H), 7.34–7.23 (m, 2H); IR (KBr): 3255, 1552, 1452, 1232, 1153, 785 cm^{-1} ; MS (ESI): m/z 358 ($\text{M} + \text{H}$) $^+$.

4.1.5.14. (Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(2,3-dimethoxyphenyl)allylidene)hydrazinecarbothioamide (7n). Yield: 62%; m.p: 132–134 $^{\circ}\text{C}$; ^1H NMR (400 MHz, DMSO-d_6):

δ 12.76 (s, 1H), 11.50 (s, 1H), 8.82 (d, J = 14.2 Hz, 1H), 8.66 (d, J = 8.3 Hz, 2H), 7.78–7.63 (m, 3H), 7.53 (d, J = 14.5 Hz, 1H), 7.30–7.20 (m, 1H), 7.18–7.03 (m, 1H), 3.83 (s, 6H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 179.0, 152.6, 149.2, 147.5, 143.3, 136.2, 135.4, 133.5, 130.3, 123.8, 123.4, 121.4, 119.4, 118.2, 115.7, 113.5, 110.9, 60.9, 55.6; IR (KBr): 3312, 2825, 1452, 1365, 1175, 958, 765 cm^{-1} ; MS (ESI): m/z 382 ($\text{M} + \text{H}$) $^+$.

4.1.5.15. *(Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(2,4,6-trimethoxyphenyl)allylidene)hydrazinecarbothioamide (7o)*. Yield: 58%; m.p: 136–138 $^{\circ}\text{C}$; ^1H NMR (400 MHz, DMSO- d_6): δ 12.89 (br s, 1H), 7.99 (br s, 1H), 7.72 (d, J = 8.0 Hz, 2H), 7.60 (d, J = 8.0 Hz, 2H), 7.43 (s, 1H), 7.34 (t, J = 14.5 Hz, 1H), 7.26 (t, J = 7.5 Hz, 1H), 6.23 (d, J = 14.1 Hz, 1H), 3.80 (s, 6H), 3.54 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 176.7, 161.1, 147.3, 146.4, 144.6, 135.3, 124.8, 122.8, 120.3, 112.3, 111.9, 56.0, 55.2; IR (KBr): 3273, 1685, 1465, 1225, 1065, 798, 745 cm^{-1} ; MS (ESI): m/z 412 ($\text{M} + \text{H}$) $^+$.

4.1.6. General procedure for the synthesis of the benzimidazolallylidenehydrazinylmethylthiazole derivatives (8a–o). To a solution of benzimidazolallylidenehydrazinecarbothioamide (7a–o) (0.1 mmol) in methanol, chloroacetone (0.11 mmol) was added at room temperature and the mixture was refluxed for 3 h (monitored by TLC). Solvent was removed under reduced pressure, and 50 mL of water was added to the residue, then extraction with chloroform was carried out (3×50 mL). Then the extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified via column chromatography on silica gel using petroleum ether/EtOAc as the eluent to get benzimidazolallylidenehydrazinylmethylthiazoles (8a–o) in good yields.

4.1.6.1. *2-((Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-phenylallylidene)hydrazinyl)-4-methylthiazole (8a)*. ^1H NMR (500 MHz, CDCl_3): Yield: 60%; m.p: 97–99 $^{\circ}\text{C}$; δ 7.54 (br s, 1H), 7.34 (d, J = 14.1 Hz, 1H), 7.29–7.23 (m, 7H), 7.22 (d, J = 14.2 Hz, 2H), 6.29 (s, 1H), 2.34 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 147.1, 137.3, 132.2, 129.5, 129.0, 128.9, 128.3, 128.1, 127.8, 127.3, 124.4, 123.2, 122.8, 17.7; IR (KBr): 3321, 2971, 1620, 1535, 1486, 1252, 732 cm^{-1} ; MS (ESI): m/z 360 ($\text{M} + \text{H}$) $^+$; HRMS (ESI): calcd for $\text{C}_{20}\text{H}_{18}\text{N}_5\text{S}$ ($\text{M} + \text{H}$) $^+$: 360.1312, found: 360.1311.

4.1.6.2. *2-((Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(p-tolyl)-allylidene)hydrazinyl)-4-methylthiazole (8b)*. Yield: 60%; m.p: 92–94 $^{\circ}\text{C}$; ^1H NMR (500 MHz, DMSO- d_6): δ 8.01 (s, 1H), 7.60 (d, J = 7.5 Hz, 2H), 7.56 (d, J = 14.5 Hz, 1H), 7.48 (t, J = 7.5 Hz, 3H), 7.42 (d, J = 7.8 Hz, 2H), 7.35 (d, J = 14.8 Hz, 2H), 7.18 (t, J = 8.5 Hz, 2H), 6.27 (s, 1H), 2.33 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3 + DMSO- d_6): δ 169.2, 147.4, 138.8, 138.4, 138.0, 133.7, 131.9, 129.37, 129.34, 127.4, 127.1, 125.6, 120.5, 113.9, 20.9, 14.2; IR (KBr): 3317, 2919, 1717, 1562, 1483, 1431, 1376, 1238, 1081, 822 cm^{-1} ; MS (ESI): m/z 374 ($\text{M} + \text{H}$) $^+$; HRMS (ESI): calcd for $\text{C}_{21}\text{H}_{20}\text{N}_5\text{S}$ ($\text{M} + \text{H}$) $^+$: 374.1482, found: 374.1484.

4.1.6.3. *2-((Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(4-methoxyphenyl)allylidene)hydrazinyl)-4-methylthiazole (8c)*. Yield: 53%; m.p: 97–99 $^{\circ}\text{C}$; ^1H NMR (500 MHz, DMSO- d_6): δ 7.85–7.83 (m, 3H), 7.70 (d, J = 8.5 Hz, 2H), 7.60 (t, J = 7.5 Hz,

2H), 7.46 (d, J = 15.2 Hz, 1H), 7.07 (d, J = 14.8 Hz, 2H), 6.56 (s, 1H), 3.85 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 161.2, 140.1, 135.2, 132.5, 132.4, 130.3, 130.1, 129.8, 127.0, 126.8, 115.2, 115.0, 114.9, 114.3, 56.2, 15.2; IR (KBr): 3315, 2932, 1612, 1542, 1483, 1368, 1250, 823 cm^{-1} ; MS (ESI): m/z 390 ($\text{M} + \text{H}$); HRMS (ESI): calcd for $\text{C}_{21}\text{H}_{20}\text{N}_5\text{OS}$ ($\text{M} + \text{H}$) $^+$: 390.1404, found: 390.1409.

4.1.6.4. *2-((Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(4-(trifluoromethoxy)phenyl)allylidene)hydrazinyl)-4-methylthiazole (8d)*. Yield: 54%; m.p: 119–121 $^{\circ}\text{C}$; ^1H NMR (400 MHz, CDCl_3): δ 7.68–7.43 (m, 4H), 7.23 (s, 1H), 7.10 (d, J = 14.6 Hz, 1H), 7.03 (d, J = 14.8 Hz, 3H), 6.88 (t, J = 7.8 Hz, 1H), 6.24 (s, 1H), 2.32 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 167.6, 147.9, 145.7, 135.3, 139.4, 128.3, 127.9, 127.3, 122.6, 120.7, 120.4, 120.2, 118.7, 117.0, 103.6, 16.6; IR (KBr): 3327, 2950, 1617, 1530, 1482, 1365, 1250, 830 cm^{-1} ; MS (ESI): m/z 444 ($\text{M} + \text{H}$) $^+$.

4.1.6.5. *2-((Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(4-fluorophenyl)allylidene)hydrazinyl)-4-methylthiazole (8e)*. Yield: 58%; m.p: 104–106 $^{\circ}\text{C}$; ^1H NMR (400 MHz, CDCl_3): δ 7.63–7.52 (m, 2H), 7.35–7.25 (m, 4H), 7.18 (d, J = 15.1 Hz, 1H), 7.13–7.01 (m, 1H), 6.96 (t, J = 14.8 Hz, 1H), 6.91–6.78 (m, 1H), 6.28 (s, 1H), 2.35 (s, 3H); IR (KBr): 2952, 1540, 1432, 1285, 1150, 982, 735 cm^{-1} ; MS (ESI): m/z 378 ($\text{M} + \text{H}$) $^+$; HRMS (ESI): calcd for $\text{C}_{20}\text{H}_{17}\text{FN}_5\text{S}$ ($\text{M} + \text{H}$) $^+$: 378.1216, found: 378.1216.

4.1.6.6. *2-((Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(4-bromophenyl)allylidene)hydrazinyl)-4-methylthiazole (8f)*. Yield: 55%; m.p: 130–132 $^{\circ}\text{C}$; ^1H NMR (400 MHz, DMSO- d_6): δ 7.63 (s, 1H), 7.57–7.40 (m, 6H), 7.34–7.20 (m, 4H), 6.33 (s, 1H), 2.25 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3 + DMSO- d_6): 166.1, 151.9, 145.9, 135.5, 131.35, 131.32, 131.2, 129.7, 128.7, 128.4, 121.0, 112.1, 21.8; IR (KBr): 2968, 1543, 1524, 1485, 1292, 1133, 972, 721 cm^{-1} ; MS (ESI): m/z 440 ($\text{M} + 2$) $^+$; HRMS (ESI): calcd for $\text{C}_{20}\text{H}_{18}\text{BrN}_5\text{S}$ ($\text{M} + 2$) $^+$: 440.0407, found: 440.0398.

4.1.6.7. *4-((1E,3Z)-3-(1H-Benzo[d]imidazol-2-yl)-3-(2-(4-methylthiazol-2-yl)hydrazono)prop-1-en-1-yl)-N,N-diethylaniline (8g)*. Yield: 52%; HPLC purity: 99.5%; m.p: 128–130 $^{\circ}\text{C}$; ^1H NMR (400 MHz, CDCl_3): δ 10.70 (br s, 1H), 7.75 (s, 1H), 7.44 (s, 1H), 7.27 (s, 3H), 7.13 (d, J = 14.8 Hz, 2H), 6.55 (d, J = 8.0 Hz, 2H), 6.15 (s, 1H), 6.48 (q, J = 15.0 Hz, 1H), 3.29 (q, J = 7.0 Hz, 4H), 1.11 (t, J = 7.2 Hz, 6H); ^{13}C NMR (125 MHz, CDCl_3 + DMSO- d_6): 163.4, 148.5, 146.6, 144.6, 143.9, 127.0, 126.5, 122.4, 111.7, 110.8, 103.2, 43.5, 16.9, 11.9; IR (KBr): 2967, 1613, 1518, 1265, 1191, 1096, 1077, 709 cm^{-1} ; MS (ESI): m/z 431 ($\text{M} + \text{H}$) $^+$; HRMS (ESI): Calcd for $\text{C}_{24}\text{H}_{27}\text{N}_6\text{S}$ ($\text{M} + \text{H}$) $^+$: 431.2057, found: 431.2048.

4.1.6.8. *2-((Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(2-chloropyridin-3-yl)allylidene)hydrazinyl)-4-methylthiazole (8h)*. Yield: 55%; m.p: 113–115 $^{\circ}\text{C}$; ^1H NMR (400 MHz, DMSO- d_6): δ 8.24 (d, J = 14.3 Hz, 1H), 8.13 (s, 1H), 7.75–7.67 (m, 2H), 7.59 (d, J = 14.8 Hz, 1H), 7.55–7.47 (m, 3H), 7.22 (m, 1H), 6.25 (s, 1H), 2.31 (s, 3H); IR (KBr): 3252, 2920, 1720, 1550, 1435, 1357, 1227, 728 cm^{-1} ; MS (ESI): m/z 395 ($\text{M} + \text{H}$) $^+$.

4.1.6.9. *2-((Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(1H-pyrrol-2-yl)allylidene)hydrazinyl)-4-methylthiazole (8i)*. Yield:

52%; m.p: 135–137 °C; ^1H NMR (400 MHz, DMSO- d_6): δ 12.87 (br s, 1H), 11.81 (br s, 1H), 7.60 (d, J = 14.2 Hz, 2H), 7.24 (s, 3H), 6.73 (s, 1H), 6.40 (s, 1H), 6.10 (d, J = 14.5 Hz, 1H), 5.98 (s, 1H), 5.78–5.64 (m, 1H), 2.30 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6): δ 164.6, 148.2, 145.2, 144.6, 129.4, 118.0, 106.9, 105.8, 104.2, 17.0; IR (KBr): 3285, 2920, 1542, 1482, 1350, 1230, 975, 832 cm^{-1} ; MS (ESI): m/z 349 ($\text{M} + \text{H}$) $^+$; HRMS (ESI): Calcd for $\text{C}_{18}\text{H}_{17}\text{N}_6\text{S}$ ($\text{M} + \text{H}$): 349.1281, found: 349.1281.

4.1.6.10. 2-((Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(1H-indol-4-yl)allylidene)hydrazinyl)-4-methyl thiazole (8j). Yield: 61%; m.p: 118–120 °C; ^1H NMR (400 MHz, CDCl_3): δ 7.56 (s, 2H), 7.35–7.24 (m, 5H), 7.17 (d, J = 14.1 Hz, 1H), 7.02 (d, J = 14.9 Hz, 1H), 6.96 (t, J = 8.5 Hz, 2H), 6.81 (t, J = 8.5 Hz, 1H), 6.27 (s, 1H), 2.34 (s, 3H); IR (KBr): 3520, 2930, 1735, 1552, 1435, 1232, 975, 732 cm^{-1} ; MS (ESI): m/z 399 ($\text{M} + \text{H}$) $^+$.

4.1.6.11. 2-((Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(4-fluoro-3-methylphenyl)allylidene)hydrazinyl)-4-methylthiazole (8k). Yield: 65%; m.p: 137–139 °C; ^1H NMR (500 MHz, CDCl_3): δ 7.51 (s, 2H), 7.31–7.21 (m, 3H), 7.12 (d, J = 14.7 Hz, 1H), 7.01 (d, J = 15.3 Hz, 1H), 6.93–6.67 (m, 2H), 6.26 (s, 1H), 2.33 (s, 3H), 2.15 (s, 3H); IR (KBr): 3252, 2975, 1735, 1535, 1235, 1085, 785 cm^{-1} ; MS (ESI): m/z 392 ($\text{M} + \text{H}$) $^+$.

4.1.6.12. 2-((Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(4-chloro-3-fluorophenyl)allylidene)hydrazinyl)-4-methylthiazole (8l). Yield: 60%; m.p: 108–110 °C; ^1H NMR (400 MHz, CDCl_3): δ 7.56 (m, 1H), 7.40–7.19 (m, 5H), 7.16–6.98 (m, 3H), 6.28 (d, J = 14.3 Hz, 1H), 2.35 (s, 3H); IR (KBr): 2850, 1635, 1535, 1185, 952, 735 cm^{-1} ; MS (ESI): m/z 412 ($\text{M} + \text{H}$); HRMS (ESI): Calcd for $\text{C}_{20}\text{H}_{16}\text{ClFN}_5\text{S}$ ($\text{M} + \text{H}$) $^+$: 412.0813, found: 412.0820.

4.1.6.13. 2-((Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(3,5-difluorophenyl)allylidene)hydrazinyl)-4-methylthiazole (8m). Yield: 58%; m.p: 102–104 °C; ^1H NMR (400 MHz, CDCl_3): δ 7.61–7.59 (m, 2H), 7.32–7.30 (m, 2H), 7.18 (s, 2H), 6.92 (d, J = 14.2 Hz, 2H), 6.70 (t, J = 15.2 Hz, 1H), 6.31 (s, 1H), 2.36 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3 + DMSO- d_6): δ 164.3, 162.3, 146.7, 140.9, 129.0, 124.8, 110.0, 109.8, 104.8, 103.4, 103.2, 103.0, 17.6; IR (KBr): 3252, 2925, 1735, 1485, 1352, 1250, 835 cm^{-1} ; MS (ESI): m/z 396 ($\text{M} + \text{H}$) $^+$; HRMS (ESI): calcd for $\text{C}_{20}\text{H}_{16}\text{F}_2\text{N}_5\text{S}$ ($\text{M} + \text{H}$) $^+$: 396.1124, found: 396.1125.

4.1.6.14. 2-((Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(2,3-dimethoxyphenyl)allylidene)hydrazinyl)-4-methylthiazole (8n). Yield: 61%; m.p: 95–97 °C; ^1H NMR (400 MHz, DMSO- d_6): δ 7.90 (s, 1H), 7.74–7.59 (m, 2H), 7.43 (d, J = 14.5 Hz, 1H), 7.32 (s, 2H), 7.21 (s, 1H), 7.10 (t, J = 7.8 Hz, 1H), 6.93 (d, J = 14.2 Hz, 1H), 6.37 (s, 1H), 3.87 (s, 6H), 2.31 (s, 3H); IR (KBr): 3225, 2923, 1432, 1235, 1132, 982, 725 cm^{-1} ; MS (ESI): m/z 420 ($\text{M} + \text{H}$) $^+$; HRMS (ESI): calcd for $\text{C}_{27}\text{H}_{23}\text{O}_2\text{N}_2\text{S}$ ($\text{M} + \text{H}$) $^+$: 420.1526, found: 420.1527.

4.1.6.15. 2-((Z)-2-((E)-1-(1H-Benzo[d]imidazol-2-yl)-3-(2,4,6-trimethoxyphenyl)allylidene)hydrazinyl)-4-methylthiazole (8o). Yield: 55%; m.p: 99–101 °C; ^1H NMR (400 MHz, DMSO- d_6): δ 12.94 (br s, 1H), 7.76–7.60 (m, 2H), 7.26 (s, 2H), 6.41 (d, J = 14.1 Hz, 1H), 6.36–6.27 (m, 3H), 6.12–6.07 (m, 1H), 3.79 (s, 9H), 2.09 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3 + DMSO- d_6): δ 171.3, 162.8, 160.7, 159.1, 148.3, 145.3, 143.8, 122.6, 108.6, 103.1, 91.2, 54.9, 54.7, 17.1; IR (KBr): 3325, 2950, 1732, 1535,

1132, 958, 832 cm^{-1} ; MS (ESI): m/z 450 ($\text{M} + \text{H}$) $^+$; HRMS (ESI): calcd for $\text{C}_{27}\text{H}_{23}\text{O}_2\text{N}_2\text{S}$ ($\text{M} + \text{H}$) $^+$: 450.1624, found: 450.1626.

4.2. Antimycobacterial evaluation assays

The unmarked autoluminescent *M. tuberculosis* H37Ra (UalRa) was homogenized with sterile glass beads in a 50 mL tube containing 5 mL of Middlebrook 7H9 medium plus 0.05% Tween 80, 10% v/v oleic acid albumin dextrose catalase (OADC) supplement (7H9-OADC-Tween80). When the OD600 value reached 0.3–0.5, the relative light unit (RLU) count was determined by putting a 1.5 mL transparent tube containing 200 μL of culture on the detection hole of the luminometer. When the RLU reached 2 million, the effective concentrations of compounds 7a–o and 8a–o were assessed over a 3-fold range increasing from 0.000001 $\mu\text{g mL}^{-1}$ to 10 $\mu\text{g mL}^{-1}$, prepared in 25 μL of UAlRa broth culture (RLU diluted to 2000–4000) grown in 7H9 broth without Tween80. In the treatment group, DMSO was used as a negative control and rifampicin (RIF; 10 $\mu\text{g mL}^{-1}$, 1 $\mu\text{g mL}^{-1}$ and 0.1 $\mu\text{g mL}^{-1}$) was used as a positive control. RLU counts were determined daily, for 5 days. In the analysis of the data, the MIClux value is the lowest drug concentration that can achieve an RLUDrug/RLUDMSO ratio of less than 10% after treatment.

4.3. MDCK cellular toxicity assays

MDCK cells were maintained in DMEM high glucose (4.5 g L^{-1}) powder medium with 10% FBS and 1% PS. Cells in the log phase were used. 500 cells per well were seeded in 384-PP plates with a 50 μL volume and the media was replaced with 25 μL after 24 h. Compounds were dissolved to 30 $\mu\text{g mL}^{-1}$ with DMSO, and a 3-fold serial dilution of the compounds from 30 $\mu\text{g mL}^{-1}$ to 0.00152 $\mu\text{g mL}^{-1}$ was performed. 75 nL of compound solution was correspondingly added using Echo520 to the 384-PP plate, and 75 nL of DMSO instead of compound solution was used as the 0% inhibitor control. After co-incubation for 72 h, 3 μL of CCK8 (5 mg mL^{-1}) was added. 2 h later at 37 °C, the plates were read in the Envision Multilabel Reader at 450 nm. The cell survival rate (activity%) was calculated with the formula: activity% = (OD experiment – OD blank)/(OD control – OD blank) $\times$ 100. The resulting data was then analyzed with the corresponding compound concentrations using Graph Pad Prism 5 Demo. The IC_{50} values were fitted using a nonlinear regression model with a sigmoidal dose response. The MDCK cells were bought from Zhongqiaoxinzhong Company, Shanghai, China.

Conflicts of interest

The authors declare there are no conflicts of interest.

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