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Synthesis and antituberculosis activity of 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazone derivatives

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ABSTRACT

New series of 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones **3a-t**, 1-methyl-5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones **4a-y** and 5-trifluoromethoxy-1-morpholinomethyl-1*H*-indole-2,3-dione 3-thiosemicarbazones **5a-m** were synthesized. The structures of the synthesized compounds were confirmed by spectral data and elemental analysis. The new 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione derivatives, along with previously synthesized 5-methyl-1*H*-indole-2,3-dione 3-thiosemicarbazones **6a-l**, were evaluated for in vitro antituberculosis activity against *Mycobacterium tuberculosis* H37Rv. 5-Methyl-1*H*-indole-2,3-dione 3-thiosemicarbazones (**3b, 3d, 3f, 6c, 6d**, and **6f**), 5-trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones (**3q–s**) and 5-trifluoromethoxy-1-morpholinomethyl-1*H*-indole-2,3-dione 3-thiosemicarbazones (**5e** and **5j–l**) were found to be the most potent inhibitors of *M. tuberculosis* growth described in this study.

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a growing global health problem because of lack of proper therapeutic agents for its remedy.[1] The human immunodeficiency virus pandemic, which contributes substantially to the morbidity and mortality from TB, and the emergence of multidrug-resistant (MDR) strains of *M. tuberculosis* have compounded the problem.[2] Although infections by drug-sensitive strains can be successfully cured, the emergence of drug resistance has prompted new drug research, particularly the search for new drug targets. When TB cases cannot be treated by first-line protocols due to resistance issues, the last resort for combating MDR infections relies on the action of second-line anti-TB drugs.[3] Among these second-line drugs for the treatment of MDR-TB, thiacetazone is a thiosemicarbazone antimicrobial that has been widely used in many developing contries.[4] Thiosemicarbazones have had a lengthy history as potential prophylactic therapeutics for human disease.[5] Recently, thiacetazone-related compounds have been synthesized and described for their activity against *M. tuberculosis*, *M. avium* and other mycobacterial species. Results indicate that SRI-224, SRI-286,[6,7] oxazolyl thiosemicarbazones[8] and some S-alkylisothiosemicarbazones[9,10] can be useful in the therapy and prophylaxis of mycobacteria infections and can represent a template for the development of novel antimycobacterial drugs (Fig. 1). However, the mechanism of action of thiacetazone and some structural analogues remain poorly understood.[7]

The discovery of the potent antimicrobial activity of 2-indolinones led in the past decade to extensive synthesis of related compounds and as a result, a variety of broad spectrum antimicrobial agents were developed. In recent years, Schiff and Mannich bases of 1*H*-indole-2,3-diones are reported to exhibit broad-spectrum chemotherapeutic properties such as anti-TB, antiviral and anticancer activities.[11–17] We described previously the anti-TB profile of 3-thiosemicarbazone, 3-phenacylisothiosemicarbazone and 3-thiazolinehydrazone derivatives of some 1*H*-2-indolinones which have exhibited a remarkable anti-TB activity.[18,19] The results reflect the effects of the various substituents introduced in the R_1, R_2 and R_3. Investigations regarding the structure–activity relationships of the tested compounds revealed that halogenation in the R_1, elongation of alkyl chain in the R_2, replacement of the alkyl in the R_2 with cyclohexyl or (non)substituted phenyl and the presence of substitution in the R_3 were efficient in increasing anti-TB activity. Moreover, R_1-nitro substituted derivatives were more active than the corresponding R_1-fluoro substituted derivatives in the 1*H*-indole-2,3-dione 3-thiosemicarbazones. The presence of the morpholine ring in some R_1-nitro substituted N-Mannich bases also seems to have a significant impact on the resultant anti-TB activity (Fig. 2).

In the light of these findings, new 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazone derivatives were synthesized in order to obtain more potent and less toxic anti-TB compounds. The structures of all the synthesized compounds were determined by analytical and spectral (IR, ^{1}H NMR, ^{13}C NMR-APT, HETCOR-2D, HSQC-2D and LCMS-APCI) methods. Log*P* (o/w)

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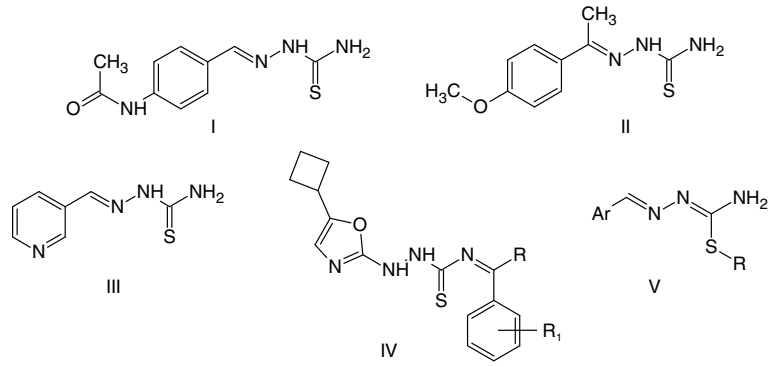


Figure 1. Structures of thiacetazone (**I**), SRI-224 (**II**), SRI-286 (**III**), oxazolyl thiosemicarbazones (**IV**) and *S*-alkylisothiosemicarbazones (**V**).

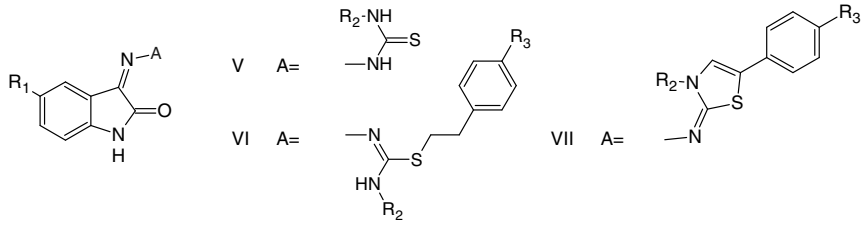
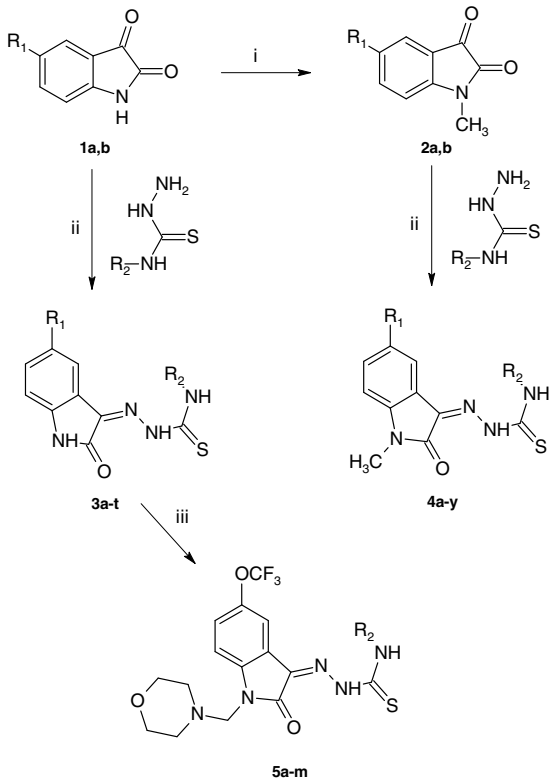


Figure. 2. Structures of 3-thiosemicarbazone (**VI**), 3-phenacylisothiosemicarbazone (**VII**) and 3-thiazolinehydrazone (**VIII**) derivatives of 1*H*-indole-2,3-diones.

values of the compounds were calculated with MOE. These new compounds, along with previously reported compounds,[20,21] were tested against *M. tuberculosis* H37Rv to construct the structure–anti-TB activity relationship.

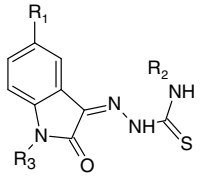


R_1= CH_3, F_3CO

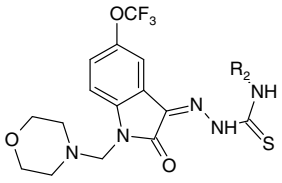
R_2= CH_3, C_2H_5, CH_2-CH=CH_2, n-C_4H_9, cyclo-C_6H_{11}, $C_6H_5CH_2$, C_6H_5, 4-$CH_3C_6H_4$, 4-$CH_3OC_6H_4$, 4-FC_6H_4, 4-ClC_6H_4, 2-BrC_6H_4, 3-BrC_6H_4, 4-BrC_6H_4, 4-$NO_2C_6H_4$

Scheme 1. Reagents and conditions: (i) NaH, CH_3I, DMF, stir, room temperature, 30 min. and reflux, 4 h (ii) EtOH, reflux, 5 h (iii) morpholine, 37% HCHO, absolute EtOH, stir, rt, 10 h.

Table 1
Log*P* values and primary in vitro antituberculosis activity screening results of **3–6**



3, **4** and **6**



5

Compound	R_1	R_2	R_3	Log*P* (o/w)	IC_{90} (μg/mL)	IC_{50} (μg/mL)	Activity
3a	CH_3	$CH_2CH{=}CH_2$	H	1,6950	>100	18.088	Weakly active
3b	CH_3	C_4H_9	H	2,4300	0.911	0.418	Active
3c	CH_3	$C_6H_5CH_2$	H	2,8210	>100	14.895	Weakly active
3d	CH_3	4-FC_6H_4	H	2,9740	1.587	1.362	Active
3e	CH_3	2-BrC_6H_4	H	3,6170	>100	2.898	Weakly active
3f	CH_3	3-BrC_6H_4	H	3,6560	1.489	1.146	Active
3g	CH_3	4-$NO_2C_6H_4$	H	2,7560	>100	>100	Inactive
3h	CF_3O	CH_3	H	2,1338	>100	1.311	Weakly active
3i	CF_3O	C_2H_5	H	2,4748	>100	0.767	Weakly active
3j	CF_3O	$CH_2CH{=}CH_2$	H	2,7958	>100	0.635	Weakly active
3k	CF_3O	C_4H_9	H	3,5308	>100	5.476	Weakly active
3l	CF_3O	cycl-C_6H_{11}	H	4,1128	>100	33.267	Weakly active
3m	CF_3O	$C_6H_5CH_2$	H	3,9218	>100	1.110	Weakly active
3n	CF_3O	C_6H_5	H	3,7878	>100	14.211	Weakly active
3o	CF_3O	4-$CH_3C_6H_4$	H	4,0858	>100	3.472	Weakly active
3p	CF_3O	4-$CH_3OC_6H_4$	H	3,7438	>100	3.339	Weakly active
3q	CF_3O	4-FC_6H_4	H	3,9408	1.546	1.333	Active
3r	CF_3O	4-ClC_6H_4	H	4,3798	1.521	1.259	Active
3s	CF_3O	4-BrC_6H_4	H	4,5858	1.530	1.341	Active
3t	CF_3O	4-$NO_2C_6H_4$	H	3,7228	>100	3.77	Weakly active
4a	CH_3	CH_3	CH_3	1,2300	>100	>100	Inactive
4b	CH_3	C_2H_5	CH_3	1,5710	>100	>100	Inactive
4c	CH_3	$CH_2CH{=}CH_2$	CH_3	1,8920	>100	>100	Inactive
4d	CH_3	C_4H_9	CH_3	2,6270	>100	>100	Inactive
4e	CH_3	$C_6H_5CH_2$	CH_3	3,0180	>100	>100	Weakly active
4f	CH_3	4-FC_6H_4	CH_3	3,0370	>100	>100	Weakly active
4g	CH_3	2-BrC_6H_4	CH_3	3,6800	>100	>100	Inactive
4h	CH_3	3-BrC_6H_4	CH_3	3,7190	>100	5.495	Weakly active
4i	CH_3	4-$NO_2C_6H_4$	CH_3	2,8190	>100	>100	Inactive
4j	CF_3O	CH_3	CH_3	2,3308	>100	>100	Inactive
4k	CF_3O	C_2H_5	CH_3	2,6718	>100	5.987	Weakly active
4l	CF_3O	$CH_2CH{=}CH_2$	CH_3	2,9928	>100	2.731	Weakly active
4m	CF_3O	C_4H_9	CH_3	3,7278	>100	40.76	Weakly active
4n	CF_3O	cycl-C_6H_{11}	CH_3	4,3098	>100	19.778	Weakly active
4o	CF_3O	$C_6H_5CH_2$	CH_3	4,1188	>100	7.299	Weakly active
4p	CF_3O	C_6H_5	CH_3	3,9848	>100	<0.2	Weakly active
4q	CF_3O	4-$CH_3C_6H_4$	CH_3	4,2828	>100	66.434	Weakly active
4r	CF_3O	4-FC_6H_4	CH_3	4,1378	>100	0.865	Weakly active
4s	CF_3O	4-ClC_6H_4	CH_3	4,5768	>100	1.706	Weakly active
4t	CF_3O	4-BrC_6H_4	CH_3	4,7828	>100	0.612	Weakly active
4y	CF_3O	4-$NO_2C_6H_4$	CH_3	3,9198	>100	>100	Inactive
5a	CF_3O	CH_3	—	1,8838	>100	1.566	Weakly active
5b	CF_3O	C_2H_5	—	2,2248	>100	2.095	Weakly active
5c	CF_3O	$CH_2CH{=}CH_2$	—	2,5458	>100	1.003	Weakly active
5d	CF_3O	C_4H_9	—	3,2808	>100	12.317	Weakly active
5e	CF_3O	cycl-C_6H_{11}	—	3,8628	5.694	1.816	Active
5f	CF_3O	$C_6H_5CH_2$	—	3,6718	>100	1.001	Weakly active
5g	CF_3O	C_6H_5	—	3,5378	>100	4.847	Weakly active
5h	CF_3O	4-$CH_3C_6H_4$	—	3,8358	>100	1.373	Weakly active
5i	CF_3O	4-$CH_3OC_6H_4$	—	3,4938	>100	2.404	Weakly active
5j	CF_3O	4-FC_6H_4	—	3,6908	3.057	2.329	Active
5k	CF_3O	4-ClC_6H_4	—	4,1298	3.041	2.468	Active
51	CF_3O	4-BrC_6H_4	—	4,3358	3.034	2.550	Active
5m	CF_3O	4-$NO_2C_6H_4$	—	3,4728	>100	6.683	Weakly active
6a	CH_3	CH_3	H	1,0330	>100	>100	Inactive
6b	CH_3	C_2H_5	H	1,3740	>100	6.962	Weakly active
6c	CH_3	cycl-C_6H_{11}	H	3,0120	0.795	0.705	Active
6d	CH_3	C_6H_5	H	2,6870	1.433	1.076	Active
6e	CH_3	4-$CH_3C_6H_4$	H	2,9850	>100	1.422	Weakly active
6f	CH_3	4-ClC_6H_4	H	3,2790	3.568	2.526	Active
6g	CH_3	4-BrC_6H_4	H	3,4850	>100	22.669	Weakly active
6h	CH_3	cycl-C_6H_{11}	CH_3	3,2090	>100	>100	Inactive
6i	CH_3	C_6H_5	CH_3	2,8840	>100	46.512	Weakly active
6j	CH_3	4-$CH_3C_6H_4$	CH_3	3,1820	>100	>100	Inactive
6k	CH_3	4-ClC_6H_4	CH_3	3,4760	>100	>100	Inactive
6l	CH_3	4-BrC_6H_4	CH_3	3,6820	>100	>100	Inactive

2. Results and discussion

2.1. Chemistry

In this study, 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione (**1a,b**) and 1-methyl-5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione (**2a,b**) reacted with different *N*-substituted thiosemicarbazides in ethanol containing a catalytic amount of sulphuric acid, to give the corresponding 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones (**3a–t**) and 1-methyl-5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones (**4a-y**). 1-Morpholinomethyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones (**5a–m**) were synthesized from the consecutive treatment of **3h-t** with formaldehyde solution and morpholine (Scheme 1).

The structures of the synthesized compounds were confirmed by elemental analyses and spectral (IR, ^{1}H NMR, ^{13}C NMR-APT, HETCOR-2D, HSQC-2D and LCMS-APCI) data. The IR spectra of **3, 4** and **5** showed absorption bands in the 3372–3160, 1703–1672 and 1238–1100 cm^{-1} regions resulting from the NH, C=O and C=S functions, respectively. ^{13}C NMR-APT of **3a, 3f, 3h, 3o, 4d, 4j, 4s, 5a** and **5h,** HETCOR spectra of **3k**, **4m** and **5d,** and HSQC spectra of **3b, 3s, 4b, 4h, 4p** and **5l** supported the IR findings and displayed signals at δ 140.45–143.28, 161.16–163.46 and 176.65–178.06 ppm which showed that there was no ^{13}C–^{1}H connection attributed to the indole C_3, indole C_2 and C=S functions. The ^{1}H NMR spectra of **3a-t** displayed the NH protons of the thiosemicarbazone moiety (δ 8.91–11.12 and 12.36–12.98 ppm) and the indole NH proton (δ 11.07–11.53 ppm) as three separate signals. The ^{1}H NMR and ^{13}C NMR spectra of **4a–y** showed two NH signals due to the thiosemicarbazone moiety (δ 8.93–11.17 and 12.27–12.91 ppm) and a signal assigned to the N-CH_3 resonance (δ 3.07–3.29 and 26.11–26.62 ppm) confirming the 1-methyl-1*H*-indole-2,3-dione 3-thiosemicarbazone structure. Observation of only two NH signals assigned to the thiosemicarbazone moiety (δ 8.86–11.15 and 12.25–12.76 ppm) and of a singlet due to N–CH_2–N function (δ 4.41–4.53 and 62.19–66.44 ppm) and of signals attributed to morpholine in the ^{1}H and ^{13}C NMR spectra of **5a–m** provided support for N-Mannich base formation. LCMS-APCI of **3a, 3b, 3d, 3f, 3i, 3l, 3o, 3r, 3s, 4b, 4d, 4h, 4k, 4n, 4p, 4s, 5b, 5e, 5h, 5k** and **5l** chosen as prototypes displayed molecular ions with different intensities.

Log*P* (o/w) values of the compounds were calculated with MOE (Table 1).

2.2. Primary antituberculosis activity

The new 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones **3a-t**, 1-methyl-5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones **4a–y** and 1-morpholinomethyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones **5a-m**, along with previously reported 5-methyl-1*H*-indole-2,3-dione 3-thiosemicarbazones **6a–l**, were evaluated against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA). The primary anti-TB screening was performed in accordance with the protocol of the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) Southern Research Institute.[22] Rifampin was used as the control drug in the tests. Compounds demonstrating a percent inhibition of bacterial growth of greater than or equal to 90% in the primary screen were retested against *M. tuberculosis* H37Rv, to determine the actual minimum inhibitory concentration (MIC) in the MABA. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 90%, relative to controls. This value was determined from the dose-response curve as the IC_{90} using a curve fitting program. Any IC_{90} value of $\leqslant$10 μg/mL was considered "Active" for antitubercular activity. Compounds active in the initial screen were tested for cytotoxicity (IC_{50}) in VERO cells. Cytotoxicity was determined from the dose–response curve as the IC_{50} using a curve fitting program. Concurrent with the determination of MICs, compounds were tested for cytotoxicity in VERO cells at concentrations 10× the MIC for *M. tuberculosis* H37Rv (Table 1). The selectivity index (SI) was defined as the ratio of the measured IC_{50} in VERO cells to the MIC (Table 2). The MIC and SI values for moving compounds into in vivo testing have to be $\leqslant$6.25 μg/mL and $\geqslant$10 (occasionally lower), respectively.

5-Methyl-1*H*-indole-2,3-dione 3-thiosemicarbazones (**3b, 3d, 3f, 6c, 6d** and **6f**), 5-trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones (**3q-s**) and 5-trifluoromethoxy-1-morpholinomethyl-1*H*-indole-2,3-dione 3-thiosemicarbazones (**5e** and **5j–l)** were found to be the most potent inhibitors of *M. tuberculosis* growth described in this study. MIC values (IC_{90}) of these compounds are 0.911, 1.587, 1.489, 0.795, 1.433, 3.568, 1.546, 1.521, 1.530, 5.694, 3.057, 3.041 and 3.034 μg/mL, respectively. However, as can be seen from Table 2, the whole of the tested compounds showed poor selectivity for mycobacteria. When these data are examined, it is observed that the anti-TB activity was increased due to elongation of the alkyl chain. Generally, replacement of the alkyl in the R_2 with cyclohexyl, phenyl, 4-fluorophenyl, 4-chlorophenyl or 4-bromophenyl has been found to yield more active compounds. The increase in activity against *M. tuberculosis* by alkyl chain elongation is related to lipophilicity as confirmed by the log*P* values of compounds (Table 1). In fact, in 5-methyl-1*H*-indole-2,3-dione-3-thiosemicarbazones, the lowest MIC value was found for the R_2-cyclohexyl substituted **6c** (0.795 μg/mL), R_2-butyl substituted **3b** (0.911 μg/mL), R_2-phenyl substituted **6d** (1.433 μg/mL) and R_2-(3-bromophenyl) substituted **3f** (1.489 μg/mL). In addition, among 5-trifluoromethoxy-1*H*-indole-2,3-dione-3-thiosemicarbazones and its N-Mannich bases, the most active compounds were **3q-s** and **5l-j** incorporating 4-fluorophenyl, 4-chlorophenyl and 4-bromophenyl at R_2. The presence of the morpholine ring in some R_1-trifluoromethoxy substituted N-Mannich bases also seems to have a significant impact on the resultant anti-TB activity. Compound **5e**, a morpholine derivative incorporating a cyclohexyl group at R_2, was found to be one of active inhibitors, whereas ligand **3l** showed weakly activity. It is interesting to note that, for the R_2-(3-bromophenyl) substituted **3f**, 5-methyl-1*H*-indole-2,3-dione 3-thiosemicarbazone derivative **3f** was substantially more active than both the R_2-(2-bromophenyl) substituted **3e** and the R_2-(4-bromophenyl) substituted **6g**. In comparison with 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones **3a–t** and **6a–l**, none of the 1-methyl-5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones **4a-t** were

Table 2
The IC_{90}, EC_{50} and SI values of some **3, 5** and **6** against *M. tuberculosis* H37Rv

Compound	R_1	R_2	R_3	IC_{90} (μg/mL)	EC_{50} (μg/mL)	SI
3b	CH_3	C_4H_9	H	0.911	4.390	4.81888
3d	CH_3	4-FC_6H_4	H	1.587	<0.195	<0.1228
3f	CH_3	3-BrC_6H_4	H	1.489	0.704	0.4728
3q	CF_3O	4-FC_6H_4	H	1.546	2.536	1.64036
3r	CF_3O	4-ClC_6H_4	H	1.521	6.391	4.20184
3s	CF_3O	4-BrC_6H_4	H	1.530	5.767	3.76928
5e	CF_3O	cycl-C_6H_{11}	—	5.694	23.437	4.11608
5j	CF_3O	4-FC_6H_4	—	3.057	2.396	0.78377
5k	CF_3O	4-ClC_6H_4	—	3.041	9.713	3.19401
5l	CF_3O	4-BrC_6H_4	—	3.034	7.453	2.45649
6c	CH_3	cycl-C_6H_{11}	H	0.795	1.572	1.97735
6d	CH_3	C_6H_5	H	1.433	1.063	0.7418
6f	CH_3	4-ClC_6H_4	H	3.568	12.361	3.4644
Rifampin	—	—	—	0.125	>100	>800

active against *M. tuberculosis*. This modification was decreased the anti-TB activity of new compounds.

3. Conclusions

New series of 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione-3-thiosemicarbazones were synthesized, their structures were confirmed by spectral data and elemental analysis. The new derivatives, along with previously reported 5-methyl-1*H*-indole-2,3-dione-3-thiosemicarbazones, were evaluated for in vitro anti-TB activity. The anti-TB screening results evidenced that many of the compounds from the series have emerged as potent anti-TB agents. The aim of this work was to reveal the correlation between structure and anti-TB activity in a series of 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones. The anti-TB activity was increased due to elongation of the alkyl chain. Generally, replacement of the alkyl in the R_2 with cyclohexyl and (non)substituted phenyl have been found to yield more active compounds. The absence of substitution at N_1 of indole ring and the increase of lipophilicity of the compounds were thought to be responsible for their high activity against *M. tuberculosis*. In conclusion, structural modification may lead to new derivatives with enhanced activity and high selectivity for mycobacteria.

4. Experimental

Melting points were estimated with a Buchi 540 melting point apparatus in open capillaries and are uncorrected. Elemental analyses were performed on a Thermo Finnigan Flash EA 1112 elemental analyzer. IR spectra were recorded on KBr discs, using a Perkin-Elmer Model 1600 FT-IR spectrometer. ^{1}H NMR, ^{13}C NMR APT, HETCOR-2D and HSQC-2D spectra were obtained on Bruker Avance DPX 400 and Varian^{UNITY} INOVA 500 spectrophotometers using DMSO-d_6. Mass spectra were determined on a Mass-AGILENT 1100 MSD instruments. All chemicals and solvents were purchased from Merck–Schuchardt, Aldrich and Fluka. Log*P* (o/w) values were calculated with MOE (version 2009.05, Chemical Computing Group, Montreal, Canada).

4.1. General method for the synthesis of *N*-substituted thiosemicarbazides

To a solution of hydrazine hydrate (5 mmol) in ethanol (10 mL), a suspension of an appropriate isothiocyanate (5 mmol) in ethanol (10 mL) was added dropwise with vigorous stirring and cooling in an ice bath. The mixture was allowed to stand overnight. The crystals formed were recrystallized from ethanol.

4.2. General method for the synthesis of 1-methyl-5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione (2a/2b)

A suspension of 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione **1a/1b** (5 mmol) and NaH (50% dispersion in mineral oil) (0.2 g) in anhydrous DMF (5 mL) was stirred for 30 min at room temperature. After addition of iodomethane (15 mmol), the mixture was refluxed for 4 h. The product was poured onto ice and water then filtered.

4.3. General method for the synthesis of 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones (3a–t)

A solution of *N*-substituted thiosemicarbazides (3.5 mmol) in ethanol (10 mL) was added to a solution of 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione **1a/1b** (3.5 mmol) in ethanol (20 mL). After addition of a drop of concentrated sulfuric acid, the mixture was refluxed on a water bath for 5 h. The product formed after cooling was filtered and washed with ethanol or recrystallized from ethanol.

4.3.1. 5-Methyl-1*H*-indole-2,3-dione 3-(*N*-allylthiosemicarbazone) (3a)

Yellow powder (78%): mp 220–221 °C; IR (KBr): υ 3335, 3289 (NH), 1696 (C=O), 1187 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 2.28 (s, 3H, 5-CH_3), 4.24 (t, J = 5.61 Hz, 2H, allyl C_1-H_2), 5.12 (dd, J = 10.49, 1.71 Hz, 1H, allyl C_3-H_{cis}), 5.18 (dd, J = 17.32, 1.71 Hz, 1H, allyl C_3-H_{trans}), 5.88–5.93 (m, 1H, allyl C_2-H), 6.80 (d, J = 7.81 Hz, 1H, indole C_6-H), 7.13 (d, J = 7.81 Hz, 1H, indole C_7-H), 7.48 (s, 1H, indole C_4-H), 9.39 (t, J = 5.61 Hz, 1H, N_4-H), 11.07 (s, 1H, indole NH), 12.56 (s, 1H, N_2-H); ^{13}C NMR (APT, DMSO-d_6/125 MHz): δ 21.07 (5-CH_3), 46.82 (allyl C_1), 111.21 (indole C_6), 116.65 (allyl C_3), 120.42 (indole C_{3a}), 121.67 (indole C_4), 131.74 (indole C_5), 131.99 (indole C_7), 132.37 (indole C_{7a}), 134.45 (allyl C_2), 140.51 (indole C_3), 163.13 (indole C_2), 177.77 (C=S); LCMS-APCI (−) *m/z* (%): 273 (MH^-, 100). Anal. Calcd for $C_{13}H_{14}N_4OS$ (274.34): C, 56.91; H, 5.14; N, 20.42. Found: C, 56.63; H, 5.38; N, 20.48.

4.3.2. 5-Methyl-1*H*-indole-2,3-dione 3-(*N*-butylthiosemicarbazone) (3b)

Yellow powder (72%): mp 179–180 °C; IR (KBr): υ 3252 (NH), 1695 (C=O), 1130 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 0.93 (t, 3H, J = 7.37 Hz, butyl C_4-H), 1.34 (hex., J = 7.44 Hz, 2H, butyl C_3-H), 1.61 (quint., J = 7.32 Hz, 2H, butyl C_2-H), 2.31 (s, 3H, 5-CH_3), 3.61 (q, J = 6.87 Hz, 2H, butyl C_1-H), 6.82 (d, J = 7.93 Hz, 1H, indole C_6-H), 7.17 (dd, J = 7.95, 0.89 Hz, 1H, indole C_7-H), 7.50 (s, 1H, indole C_4-H), 9.26 (t, J = 5.82 Hz, 1H, N_4-H), 11.10 (s, 1H, indole NH), 12.53 (s, 1H, N_2-H); ^{13}C NMR (HSQC-2D, DMSO-d_6/125 MHz): δ 14.22 (butyl C_4), 20.06 (butyl C_3), 21.06 (5-CH_3), 31.03 (butyl C_2), 44.33 (butyl C_1), 111.21 (indole C_6), 120.46 (indole C_{3a}), 121.61 (indole C_4), 131.72 (indole C_5), 131.93 (indole C_7), 132.13 (indole C_{7a}), 140.45 (indole C_3), 163.14 (indole C_2), 177.34 (C=S); LCMS-APCI (−/+) *m/z* (%): *m/z* 289 (MH^-, 100), 291 (MH^+, 58), 282 (100). Anal. Calcd for $C_{14}H_{18}N_4OS$ (290.38): C, 57.91; H, 6.25; N, 19.29. Found: C, 57.31; H, 6.26; N, 19.70.

4.3.3. 5-Methyl-1*H*-indole-2,3-dione 3-(*N*-benzylthiosemicarbazone) (3c)

Orange crystals (93%): mp 236–237 °C; IR (KBr): υ 3367, 3160 (NH), 1686 (C=O), 1142 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 2.27 (s, 3H, 5-CH_3), 4.86 (d, 2H, J = 6.34 Hz, benzyl CH_2), 6.80 (d, J = 8.29 Hz, 1H, indole C_6-H), 7.14 (d, J = 7.81 Hz, 1H, indole C_7-H), 7.24 (t, J = 7.32 Hz, 1H, benzyl C_4-H), 7.31–7.36 (m, 4H, benzyl $C_{2,3,5,6}$-H), 7.47 (s, 1H, indole C_4-H), 9.77 (t, J = 6.34 Hz, 1H, N_4-H), 11.08 (s, 1H, indole NH), 12.62 (s, 1H, N_2-H). Anal. Calcd for $C_{17}H_{16}N_4OS$ (324.40): C, 62.94; H, 4.97; N, 17.27. Found: C, 62.12; H, 4.72; N, 17.15.

4.3.4. 5-Methyl-1*H*-indole-2,3-dione 3-[*N*-(4-fluorophenyl) thiosemicarbazone] (3d)

Orange powder (86%): mp 229–230 °C; IR (KBr): υ 3303, 3207 (NH), 1689 (C=O), 1163 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 2.31 (s, 3H, 5-CH_3), 6.83 (d, J = 7.94 Hz, 1H, indole C_6-H), 7.19 (dd, J = 7.95, 0.95 Hz, 1H, indole C_7-H), 7.27 (t, J = 7.74 Hz, 2H, phenyl $C_{2,6}$-H), 7.59–7.62 (m, 3H, indole C_4-H and phenyl $C_{3,5}$-H), 10.81 (s, 1H, N_4-H), 11.16 (s, 1H, indole NH), 12.78 (s, 1H, N_2-H); LCMS-APCI (+) m/z (%): 329 (MH^+, 100). Anal. Calcd for $C_{16}H_{13}FN_4OS$ (328.36): C, 58.52; H, 3.99; N, 17.06. Found: C, 58.32; H, 4.02; N, 17.23.

4.3.5. 5-Methyl-1*H*-indole-2,3-dione 3-[*N*-(2-bromophenyl) thiosemicarbazone] (3e)

Orange powder (99%): mp 249 °C; IR (KBr): υ 3207 (NH), 1689 (C=O), 1134 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 2.48 (s, 3H, 5-CH_3), 6.82 (d, J = 7.81 Hz, 1H, indole C_6-H), 7.17 (dd, J = 7.81,

0.97 Hz, 1H, indole C_7-H), 7.29 (td, J = 7.56, 1.47 Hz, 1H, phenyl C_4-H), 7.45 (td, J = 7.56, 1.46 Hz, 1H, phenyl C_5-H), 7.54–7.57 (m, 2H, phenyl C_3-H and indole C_4-H), 7.73 (dd, J = 7.81, 0.97 Hz, 1H, phenyl C_6-H), 10.77 (s, 1H, N_4-H), 11.13 (s, 1H, indole NH), 12.80 (s, 1H, N_2-H). Anal. Calcd for $C_{16}H_{13}BrN_4OS$ (389.26): C, 49.37; H, 3.37; N, 14.39. Found: C, 49.51; H, 3.38; N, 14.36.

4.3.6. 5-Methyl-1*H*-indole-2,3-dione 3-[*N*-(3-bromophenyl) thiosemicarbazone] (3f)

Yellow powder (72%): mp 236–238 °C; IR (KBr): υ 3310 (NH), 1687 (C=O), 1145 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 2.31 (s, 3H, 5-CH_3), 6.84 (d, J = 7.91 Hz, 1H, indole C_6-H), 7.19 (d, J = 8.08 Hz, 1H, indole C_7-H), 7.39 (t, J = 8.00 Hz, 1H, phenyl C_5-H), 7.47 (d, J = 8.27 Hz, 1H, phenyl C_6-H), 7.61 (s, 1H, indole C_4-H), 7.71 (d, J = 8.00 Hz, 1H, phenyl C_4-H), 7.91 (s, 1H, phenyl C_2-H), 10.86 (s, 1H, N_4-H), 11.18 (s, 1H, indole NH), 12.84 (s, 1H, N_2-H); ^{13}C NMR (APT-DMSO-d_6/125 MHz): δ 21.10 (5-CH_3), 111.34 (indole C_6), 120.28 (indole C_{3a}), 121.21 (phenyl C_3), 122.25 (indole C_4), 124.82 (phenyl C_6), 128.24 (phenyl C_4), 129.08 (phenyl C_2), 130.65 (phenyl C_5), 131.87 (indole C_5), 132.44 (indole C_7), 133.24 (indole C_{7a}), 140.52 (phenyl C_1), 140.85 (indole C_3), 163.22 (indole C_2), 176.65 (C=S); LCMS-APCI (+) m/z (%): 385, 387 (100, 17), 383, 385 (83, 100). Anal. Calcd for $C_{16}H_{13}BrN_4OS$ (389.26): C, 49.37; H, 3.37; N, 14.39. Found: C, 49.38; H, 3.04; N, 14.41.

4.3.7. 5-Methyl-1*H*-indole-2,3-dione 3-[*N*-(4-nitrophenyl) thiosemicarbazone] (3g)

Red powder (73%): mp 262–263 °C; IR (KBr): υ 3193 (NH), 1694 (C=O), 1177 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 2.30 (s, 3H, 5-CH_3), 6.83 (d, J = 8.30 Hz, 1H, indole C_6-H), 7.19 (d, J = 7.32 Hz, 1H, indole C_7-H), 7.60 (s, 1H, phenyl C_4-H), 8.07 (d, J = 8.79 Hz, 2H, phenyl $C_{2,6}$-H), 8.28 (dd, J = 6.84, 1.95 Hz, 2H, phenyl $C_{3,5}$-H), 11.06 (s, 1H, N_4-H), 11.17 (s, 1H, indole NH), 12.98 (s, 1H, N_2-H). Anal. Calcd for $C_{16}H_{13}N_5O_3S$ (355.37): C, 54.08; H, 3.69; N, 19.71. Found: C, 54.30; H, 3.73; N, 20.15.

4.3.8. 5-Trifluoromethoxy-1*H*-indole-2,3-dione 3-(*N*-methyl-thiosemicarbazone) (3h)

Yellow powder (80%): mp 234 °C; IR (KBr): υ 3295 (NH), 1699 (C=O), 1142 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 3.10 (d, J = 4.6 Hz, 3H, CH_3), 7.02 (d, J = 8.50 Hz, 1H, indole C_7-H), 7.36 (dd, J = 8.50, 1.80 Hz, 1H, indole C_6-H), 7.63 (d, J = 1.40 Hz, 1H, indole C_4-H), 9.39 (q, J = 4.50 Hz, 1H, N_4-H), 11.37 (s, 1H, indole NH), 12.46 (s, 1H, N_2-H); ^{13}C NMR (APT, DMSO-d_6,/125 MHz): δ 31.73 (CH_3), 112.52 (indole C_7), 114.18 (indole C_4), 118.11 (q, J = 255.72, CF_3O), 121.93 (indole C_{3a}), 124.26 (indole C_6), 130.88 (indole C_{7a}), 141.50 (indole C_3), 143.97 (indole C_5), 163.12 (indole C_2), 178.06 (C=S). Anal. Calcd for $C_{11}H_9F_3N_4O_2S$ (318.27): C, 41.51; H, 2.85; N, 17.60. Found: C, 42.28; H, 2.53; N, 17.97.

4.3.9. 5-Trifluoromethoxy-1*H*-indole-2,3-dione 3-(*N*-ethylthio-semicarbazone) (3i)

Yellow crystals (67%): mp 235–236 °C; IR (KBr): υ 3324, 3182 (NH), 1696 (C=O), 1155 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 1.21 (t, J = 7.10 Hz, 3H, ethyl CH_3), 3.65 (p, J = 7.0 Hz, 2H, ethyl CH_2), 7.02 (d, J = 8.50, Hz, 1H, indole C_7-H), 7.36 (dd, J = 8.50, 2.30 Hz, 1H, indole C_6-H), 7.66 (d, J = 1.70 Hz, 1H, indole C_4-H), 9.41 (t, J = 5.60 Hz, 1H, N_4-H), 11.36 (s, 1H, indole NH), 12.43 (s, 1H, N_2-H); LCMS-APCI (−/+): m/z (%) 333 (MH^+, 100), 331 (MH^-, 87), 230 (100). Anal. Calcd for $C_{12}H_{11}F_3N_4O_2S$ (332.30): C, 43.37; H, 3.34; N, 16.86. Found: C, 43.34; H, 2.81; N, 16.82.

4.3.10. 5-Trifluoromethoxy-1*H*-indole-2,3-dione 3-(*N*-allylthio-semicarbazone) (3j)

Orange crystals (74%): mp 224–226 °C; IR (KBr): υ 3228 (NH), 1698 (C=O), 1172 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 4.26 (t, J = 5.49 Hz, 2H, allyl C_1-H), 5.14 (dd, J = 10.22, 1.37 Hz, 1H, allyl C_3-H_{cis}), 5.19 (dd, J = 17.23, 1.67 Hz, 1H, allyl C_3-H_{trans}), 5.87–5.93 (m, 1H, allyl C_2-H), 7.00 (d, J = 8.54 Hz, 1H, indole C_7-H), 7.33 (dd, J = 8.54, 1.53 Hz, 1H, indole C_6-H), 7.66 (d, J = 1.53 Hz, 1H, indole C_4-H), 9.54 (t, J = 5.79 Hz, 1H, N_4-H), 11.53 (s, 1H, indole NH), 12.47 (s, 1 H, N_2-H). Anal. Calcd for $C_{13}H_{11}F_3N_4O_2S$ (344.31): C, 45.35; H, 3.22; N, 16.27. Found: C, 45.98; H, 2.95; N, 16.33.

4.3.11. 5-Trifluoromethoxy-1 *H*-indole-2,3-dione 3-(*N*-n-butyl-thiosemicarbazone) (3k)

Yellow powder (87%): mp 234 °C; IR (KBr): υ 3257 (NH), 1695 (C=O), 1158 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 0.92 (t, J = 7.30 Hz, 3H, butyl C_4-H), 1.31–1.37 (m, 2H, butyl C_3-H), 1.62 (p, J = 7.40 Hz, 2H, butyl C_2-H), 3.62 (q, J = 6.80 Hz, 2H, butyl C_1-H), 7.00 (d, J = 8.50, Hz, 1H, indole C_7-H), 7.34 (dd, J = 8.60, 2.40 Hz, 1 H, indole C_6-H), 7.66 (d, J = 1.70 Hz, 1H, indole C_4-H), 9.37 (t, J = 5.90 Hz, 1H, N_4-H), 11.35 (s, 1H, indole NH), 12.44 (s, 1H, N_2-H); ^{13}C NMR (HETCOR-2D, DMSO-d_6/125 MHz): δ 14.19 (butyl C_4), 20.05 (butyl C_3), 30.98 (butyl C_2), 44.38 (butyl C_1), 112.55 (indole C_7), 114.43 (indole C_4), 120.68 (q, J = 258.10, CF_3O), 121.97 (indole C_{3a}), 124.34 (indole C_6), 131.00 (indole C_{7a}), 141.56 (indole C_3), 143.97 (indole C_5), 163.16 (indole C_2), 177.33 (C=S). Anal. Calcd for $C_{14}H_{15}F_3N_4O_2S$ (360.35): C, 46.66; H, 4.20; N, 15.55. Found: C, 46.94; H, 3.82; N, 15.67.

4.3.12. 5-Trifluoromethoxy-1*H*-indole-2,3-dione 3-(*N*-cyclohe-xylthiosemicarbazone) (3l)

Yellow powder (75%): mp 249 °C; IR (KBr): υ 3262 (NH), 1696 (C=O), 1162 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 1.13–1.94 (m, 10H, cycl. $C_{2,3,4,5,6}$-H), 4.18–4.24 (m, 1H, cycl. C_1-H), 7.01 (d, J = 8.50 Hz, 1H, indole C_7-H), 7.35, 7.37 (2xdd, J = 8.50, 2.40 Hz, 1H, indole C_6-H), 7.76 (d, J = 2.40 Hz, 1H, indole C_4-H), 8.91 (d, J = 8.50 Hz, 1H, N_4-H), 11.37 (s, 1H, indole NH), 12.49 (s, 1H, N_2-H); LCMS-APCI (−/+): m/z (%) 387 (MH^+, 100), 385 (MH^-, 6), 265 (100). Anal. Calcd for $C_{16}H_{17}F_3N_4O_2S$ (386.39): C, 49.73; H, 4.43; N, 14.50. Found: C, 49.37; H, 4.30; N, 14.46.

4.3.13. 5-Trifluoromethoxy-1*H*-indole-2,3-dione-3-(*N*-benzyl-thiosemicarbazone) (3m)

Orange crystals (90%): mp 215–216 °C; IR (KBr): υ 3221 (NH), 1702 (C=O), 1238 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 4.88 (d, J = 4.10 Hz, 2H, benzyl CH_2), 7.00 (d, J = 8.54 Hz, 1H, indole C_7-H), 7.25 (t, J = 6.40 Hz, 1H, benzyl C_4-H), 7.33–7.36 (m, 5H, indole C_6-H, benzyl $C_{2,3,4,5,6}$-H), 7.64 (d, J = 1.83 Hz, 1H, indole C_4-H), 9.90 (t, J = 6.25 Hz, 1H, N_4-H), 11.35 (s, 1H, indole NH), 12.53 (s, 1H, N_2-H). Anal. Calcd for $C_{17}H_{13}F_3N_4O_2S.1/2H_2O$ (403.38): C, 50.61; H, 3.49; N, 13.89. Found: C, 50.72; H, 3.36; N, 13.61.

4.3.14. 5-Trifluoromethoxy-1*H*-indole-2,3-dione-3-(*N*-phenyl-thiosemicarbazone) (3n)

Orange powder (99%): mp 229–232 °C; IR (KBr): υ 3316, 3282 (NH), 1698 (C=O), 1171 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 7.02 (d, J = 8.54 Hz, 1H, indole C_7-H), 7.28 (t, J = 7.32 Hz, 1H, phenyl C_4-H), 7.35 (dd, J = 8.54, 2.44 Hz, 1H, indole C_6-H), 7.43 (t, J = 7.32 Hz, 2H, phenyl $C_{3,5}$-H), 7.58 (d, J = 7.32 Hz, 2H, phenyl $C_{2,6}$-H), 7.79 (d, J = 1.83 Hz, 1H, indole C_4-H), 10.87 (s, 1H, N_4-H), 11.38 (s, 1H, indole NH), 12.64 (s, 1H, N_2-H). Anal. Calcd for $C_{16}H_{11}F_3N_4O_2S$ (380.34): C, 50.53; H, 2.92; N, 14.73. Found: C, 50.38; H, 2.83; N, 14.76.

4.3.15. 5-Trifluoromethoxy-1*H*-indole-2,3-dione 3-[*N*-(4-methyl-phenyl)thiosemicarbazone] (3o)

Orange crystals (88%): mp 230–233 °C; IR (KBr): υ 3307 (NH), 1697 (C=O), 1153 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 2.34 (s, 3H, CH_3), 7.03 (d, J = 8.50 Hz, 1H, indole C_7-H), 7.24 (d, J = 8.10 Hz, 2H, phenyl $C_{3,5}$-H), 7.37 (dd, J = 8.50, 1.70 Hz, 1H, indole C_6-H), 7.46 (d, J = 8.30 Hz, 2H, phenyl $C_{2,6}$-H), 7.67 (d, J = 1.40 Hz,

1H, indole C_4-H), 10.66 (s, 1H, N_4-H), 11.20 (s, 1H, indole NH), 12.36 (s, 1H, N_2-H); ^{13}C NMR (APT DMSO-d_6/125 MHz): δ 21.32 (CH_3), 112.86 (indole C_7), 115.17 (indole C_4), 120.91 (q, J = 255.45 Hz, CF_3O), 122.16 (indole C_{3a}), 124.82 (indole C_6), 126.38 (phenyl C_3, C_5), 129.61 (phenyl C_2, C_6),131.75 (indole C_{7a}), 136.30 (phenyl C_4), 136.44 (phenyl C_1), 142.10 (indole C_3), 144.29 (indole C_5), 163.46 (indole C_2), 177.10 (C=S); LCMS-APCI (−/+): *m/z* (%) 395 (MH^+, 100), 393 (MH^-, 100). Anal. Calcd for $C_{17}H_{13}F_3N_4O_2S$ (394.37): C, 51.77; H, 3.32; N, 14.21. Found: C, 51.71; H, 3.06; N, 14.39.

4.3.16. 5-Trifluoromethoxy-1*H*-indole-2,3-dione 3-[*N*-(4-methoxyphenyl)thiosemicarbazone] (3p)

Orange powder (89%): mp 228–229 °C; IR (KBr): υ 3266 (NH), 1701 (C=O), 1154 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 3.77 (s, 3H, OCH_3), 6.98 (d, J = 9.16 Hz, 2H, phenyl $C_{3,5}$-H), 7.01 (d, J = 8.24 Hz, 1H, indole C_7-H),), 7.35 (dd, J = 8.54, 2.30 Hz, 1H, indole C_6-H), 7.44 (d, J = 9.15 Hz, 2H, phenyl C_2, $_6$-H), 7.78 (br s, 1H, indole C_4-H), 10.78 (s, 1H, N_4-H), 11.37 (s, 1H, indole NH), 12.59 (s, 1H, N_2-H). Anal. Calcd for $C_{17}H_{13}F_3N_4O_3S$ (410.37): C, 49.76; H, 3.19; N, 13.65. Found: C, 49.40; H, 3.31; N, 13.88.

4.3.17. 5-Trifluoromethoxy-1*H*-indole-2,3-dione 3-[*N*-(4-fluorophenyl)thiosemicarbazone] (3q)

Orange powder (84%): mp 224–227 °C; IR (KBr): υ 3291 (NH), 1690 (C=O), 1154 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 7.01 (d, J = 8.54 Hz, 1H, indole C_7-H), 7.26 (t, J = 8.84 Hz, 2H, phenyl $C_{3,5}$-H), 7.35 (dd, J = 8.54, 2.44 Hz, 1H, indole C_6-H), 7.58 (dd, J = 8.84, 4.88 Hz, 2H, phenyl $C_{2,6}$-H), 7.76 (br s, 1H, indole C_4-H), 10.87 (s, 1H, N_4-H), 11.39 (s, 1H, indole NH), 12.65 (s, 1H, N_2-H). Anal. Calcd for $C_{16}H_{10}F_4N_4O_2S$ (398.33): C, 48.24; H, 2.53; N, 14.07. Found: C, 48.86; H, 2.45; N, 14.21.

4.3.18. 5-Trifluoromethoxy-1*H*-indole-2,3-dione 3-[*N*-(4-chlorophenyl)thiosemicarbazone] (3r)

Orange crystals (77%): mp 233–234 °C; IR (KBr): υ 3300 (NH), 1698 (C=O), 1157 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 6.94 (d, J = 8.50 Hz, 1H, indole C_7-H), 7.28 (dd, J = 8.50, 1.70 Hz, 1H, indole C_6-H), 7.41 (d, J = 8.70 Hz, 2H, phenyl $C_{3,5}$-H), 7.56 (d, J = 8.70 Hz, 2H, phenyl $C_{2,6}$-H), 7.69 (d, J = 1.50 Hz, 1H, indole C_4-H), 10.83 (s, 1H, N_4-H), 11.33 (s, 1H, indole NH), 12.61 (s, 1H, N_2-H); LCMS-APCI (−/+): *m/z* (%) 415, 417 [(MH^+, MH^++2) 100, 39], 413, 415 [(MH^-, MH^-+2) 100, 51], 230 (79). Anal. Calcd for $C_{15}H_{10}ClF_3N_4O_2S$ (414.78): C, 46.33; H, 2.43; N, 13.51. Found: C, 46.49; H, 2.07; N, 13.76.

4.3.19. 5-Trifluoromethoxy-1*H*-indole-2,3-dione 3-[*N*-(4-bromophenyl)thiosemicarbazone] (3s)

Orange crystals (95%): mp 238–239 °C; IR (KBr): υ 3302 (NH), 1698 (C=O), 1161 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 7.02 (d, J = 8.29 Hz, 1H, indole C_7-H), 7.35 (dd, J = 8.29, 2.44 Hz, 1H, indole C_6-H), 7.57–7.62 (m, 4H, phenyl $C_{2,3,5,6}$-H), 7.77 (d, J = 1.95 Hz, 1H, indole C_4-H), 10.88 (s, 1H, N_4-H), 11.39 (s, 1H, indole NH), 12.69 (s, 1H, N_2-H); ^{13}C NMR (HSQC-2D, DMSO-d_6/125 MHz): δ 112.94 (indole C_7), 117.19 (indole C_4), 119.31 (phenyl C_4), 120.91 (q, J = 255.93 Hz, CF_3O), 122.03 (indole C_{3a}), 124.99 (indole C_6), 128.42 (phenyl C_3, C_5), 132.02 (phenyl C_2, C_6), 132.21 (indole C_{7a}), 138.41 (phenyl C_1), 142.12 (indole C_3), 144.31 (indole C_5), 163.46 (indole C_2), 177.10 (C=S); LCMS-APCI (−): *m/z* (%) 457, 459 [(MH^-, MH^-+2) 97, 100]. Anal. Calcd for $C_{16}H_{10}BrF_3N_4O_2S$ (459.24): C, 41.85; H, 2.19; N, 12.20. Found: C, 42.38; H, 2.06; N, 12.73.

4.3.20. 5-Trifluoromethoxy-1*H*-indole-2,3-dione-3-[*N*-(4-nitrophenyl)thiosemicarbazone] (3t)

Orange powder (60%): mp 246–247 °C; IR (KBr): υ 3283 (NH), 1698 (C=O), 1151 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 7.03 (d, J = 8.54 Hz, 1H, indole C_7-H), 7.38 (dd, J = 8.54, 1.83 Hz, 1H, indole C_6-H), 7.77 (br s, 1H, indole C_4-H), 8.06 (d, J = 9.15 Hz, 2H, phenyl C_2, $_6$-H), 8.30 (d, J = 9.15 Hz, 2H, phenyl C_3, $_5$-H), 11.12 (s, 1H, N_4-H), 11.44 (s, 1H, indole NH), 12.85 (s, 1H, N_2-H). Anal. Calcd for $C_{16}H_{10}F_3N_5O_4S$ (425.34): C, 45.18; H, 2.37; N, 16.47. Found: C, 45.08; H, 2.26; N, 16.39.

4.4. General method for the synthesis of 1-methyl-5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazones (4a–y)

A solution of *N*-substituted thiosemicarbazides (3.5 mmol) in ethanol (10 mL) was added to a solution of 1-methyl-5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione **2a/2b** (3.5 mmol) in ethanol (20 mL). After addition of a drop of concentrated sulfuric acid, the mixture was refluxed on a water bath for 5 h. The product formed after cooling was filtered and washed with ethanol or recrystallized from ethanol.

4.4.1. 1,5-Dimethyl-1*H*-indole-2,3-dione 3-(*N*-methylthiosemicarbazone) (4a)

Yellow powder (88%): mp 259–60 °C; IR(KBr): υ 3238 (NH), 1683 (C=O), 1100 (C=S). ^{1}H NMR (DMSO-d_6/500 MHz): δ 2.31 (s, 3H, 5-CH_3), 3.07 (d, J = 4.39 Hz, 3H, N-CH_3), 3.17 (s, 3H, N-CH_3), 7.00 (d, J = 8.29 Hz, 1H, indole C_6-H), 7.23 (d, J = 7.80 Hz, 1H, indole C_7-H), 7.48 (s, 1H, indole C_4-H), 9.22 (s, 1H, N_4-H), 12.47 (s, 1H, N_2-H). Anal. Calcd for $C_{12}H_{14}N_4OS$ (262.33): C, 54.94; H, 5.38; N, 21.36. Found: C, 54.73; H, 5.07; N, 21.28.

4.4.2. 1,5-Dimethyl-1*H*-indole-2,3-dione 3-(*N*-ethylthiosemicarbazone) (4b)

Yellow powder (80%): mp 250–251 °C; IR(KBr): υ 3301 (NH), 1686 (C=O), 1100 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 1.20 (t, J = 7.14 Hz, 3H, ethyl C_2-H), 2.33 (s, 3H, 5-CH_3), 3.20 (s, 3H, N-CH_3), 3.65 (quin., J = 6.61 Hz, 2H, ethyl C_1-H), 7.02 (d, J = 8.00 Hz, 1H, indole C_6-H), 7.25 (dd, J = 7.98, 0.88 Hz, 1H, indole C_7-H), 7.52 (s, 1H, indole C_4-H), 9.31 (t, J = 5.80 Hz, 1H, N_4-H), 12.45 (s, 1H, N_2-H); ^{13}C NMR (HSQC-2D, DMSO-d_6/125 MHz): δ 14.51 (ethyl C_2), 21.08 (5-CH_3), 26.13 (N-CH_3), 39.36–40.61 (DMSO, ethyl C_1), 110.00 (indole C_6), 119.69 (indole C_{3a}), 121.33 (indole C_4), 131.32 (indole C_5), 131.77 (indole C_7), 132.39 (indole C_{7a}), 141.78 (indole C_3), 161.19 (indole C_2), 177.09 (C=S); LCMS-APCI (+) *m/z* (%): 277 (MH^+, 100). Anal. Calcd for $C_{13}H_{16}N_4OS$ (276.35): C, 56.50; H, 5.84; N, 20.27. Found: C, 56.46; H, 5.41; N, 20.48.

4.4.3. 1,5-Dimethyl-1*H*-indole-2,3-dione 3-(*N*-allylthiosemicarbazone) (4c)

Orange powder (67%): mp 208–209 °C; IR(KBr): υ 3266 (NH), 1687 (C=O), 1186 (C=S). ^{1}H NMR (DMSO-d_6/500 MHz): δ 2.31 (s, 3H, 5-CH_3), 3.17 (s, 3H, N-CH_3), 4.24 (td, J = 5.80, 1.47 Hz, 2H, allyl C_1-H), 5.13 (dd, J = 7.32, 1.46 Hz, 1H, allyl C_3-H_{cis}), 5.19 (dd, J = 15.61, 1.46 Hz, 1H, allyl C_3-H_{trans}), 5.89–5.94 (m, 1H, allyl C_2-H), 6.99 (d, J = 7.57 Hz, 1H, indole C_6-H), 7.22 (d, J = 8.29 Hz, 1H, indole C_7-H), 7.50 (s, 1H, indole C_4-H), 9.42 (t, J = 5.80 Hz, 1H, N_4-H), 12.49 (s, 1H, N_2-H). Anal. Calcd for $C_{14}H_{16}N_4OS$ (288.36): C, 58.31; H, 5.59; N, 19.43. Found: C, 58.37; H, 5.42; N, 19.60.

4.4.4. 1,5-Dimethyl-1*H*-indole-2,3-dione 3-(*N*-butylthiosemicarbazone) (4d)

Orange crystals (69%): mp 201–203 °C; IR(KBr): υ 3281 (NH), 1685 (C=O), 1173 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 0.93 (t, J = 7.36 Hz, 3H, butyl C_4-H), 1.34 (hex., J = 7.45 Hz, 2H, butyl C_3-H), 1.61 (pent, J = 7.45 Hz, 2H, butyl C_2-H), 2.34 (s, 3H, 5-CH_3), 3.20 (s, 3H, N-CH_3), 3.61 (q, J = 6.86 Hz, 2 H, butyl C_1-H), 7.04 (d, J = 8.00 Hz, 1H, indole C_6-H), 7.26 (dd, J = 8.04, 0.81 Hz, 1H, indole C_7-H), 7.54 (s, 1H, indole C_4-H), 9.29 (t, J = 5.84 Hz, 1 H, N_4-H), 12.47 (s, 1H, N_2-H); ^{13}C NMR (APT, DMSO-d_6/125 MHz): δ 14.24

(butyl C_4), 20.06 (butyl C_3), 21.07 (5-CH_3), 26.11 (N-CH_3), 31.02 (butyl C_2), 44.37 (butyl C_1), 109.97 (indole C_6), 119.69 (indole C_{3a}), 121.34 (indole C_4), 131.29 (indole C_5), 131.75 (indole C_7), 132.37 (indole C_{7a}), 141.76 (indole C_3), 161.18 (indole C_2), 177.32 (C=S); LCMS-APCI (+) *m/z* (%): 305 (MH^+, 56), 282 (96), 79 (99), 65 (100). Anal. Calcd for $C_{15}H_{20}N_4OS$ (304.41): C, 59.18; H, 6.62; N, 18.41. Found: C, 59.44; H, 6.79; N, 18.70.

4.4.5. 1,5-Dimethyl-1*H*-indole-2,3-dione 3-(*N*-benzylthiosemicarbazone) (4e)

Orange powder (79%): mp 191–192 °C; IR(KBr): υ 3287 (NH), 1687 (C=O), 1172 (C=S); ^{1}H NMR (DMSO/500 MHz): 2.29 (s, 3H, 5-CH_3), 3.16 (s, 3H, N-CH_3), 4.87 (d, *J* = 5.85 Hz, 2H, benzyl CH_2), 6.96 (d, *J* = 7.81 Hz, 1H, indole C_6-H), 7.20 (d, *J* = 8.30 Hz, 1H, indole C_7-H), 7.25 (t, *J* = 7.32 Hz, 1H, benzyl C_4-H), 7.32–7.37 (m, 4H, benzyl $C_{2,3,5,6}$-H), 7.47 (s, 1H, indole C_4-H), 9.78 (t, *J* = 5.86 Hz, 1H, N_4-H), 12.53 (s, 1H, N_2-H). Anal. Calcd for $C_{18}H_{18}N_4OS$ (338.42): C, 63.88; H, 5.36; N, 16.56. Found: C, 63.23; H, 5.48; N, 16.48.

4.4.6. 1,5-Dimethyl-1*H*-indole-2,3-dione 3-[*N*-(4-fluorophenyl) thiosemicarbazone] (4f)

Orange powder (81%): mp 236 °C; IR(KBr): υ 3208 (NH), 1675 (C=O), 1161 (C=S); ^{1}H NMR (DMSO/400 MHz): 2.34 (s, 3H, 5-CH_3), 3.21 (s, 3H, N-CH_3), 7.05 (d, *J* = 8.00 Hz, 1H, indole C_6-H), 7.25–7.29 (m, 3H, indole C_7-H and phenyl $C_{2,6}$-H), 7.59–7.63 (m, 3H, indole C_4-H and phenyl $C_{3,5}$-H), 10.84 (s, 1H, N_4-H), 12.71 (s, 1H, N_2-H). Anal. Calcd for $C_{17}H_{15}FN_4OS$ (342.39): C, 59.63; H, 4.42; N, 16.36. Found: C, 59.66; N, 4.36; N, 16.54.

4.4.7. 1,5-Dimethyl-1*H*-indole-2,3-dione 3-[*N*-(2-bromophenyl) thiosemicarbazone] (4g)

Orange powder (85%): mp 231–232 °C; IR(KBr): υ 3234 (NH), 1682 (C=O), 1162 (C=S). ^{1}H NMR (DMSO-d_6/500 MHz): δ 2.32 (s, 3H, 5-CH_3), 3.20 (s, 3H, N-CH_3), 7.04 (d, *J* = 7.81 Hz, 1H, indole C_6-H), 7.26 (d, *J* = 7.32 Hz, 1H, indole C_7-H), 7.30 (td, *J* = 7.81, 1.46 Hz, 1H, phenyl C_4-H), 7.45 (t, *J* = 7.81 Hz, 1H, phenyl C_5-H), 7.54 (d, *J* = 7.81 Hz, 1H, phenyl C_3-H), 7.58 (s, 1H, indole C_4-H), 7.74 (d, *J* = 8.29 Hz, 1H, phenyl C_6-H), 10.80 (s, 1H, N_4-H), 12.73 (s, 1H, N_2-H). Anal. Calcd for $C_{17}H_{15}BrN_4OS.1/2H_2O$ (412.29): C, 49.52; H, 3.91; N, 13.58. Found: C, 49.62; N, 3.92; N, 14.17.

4.4.8. 1,5-Dimethyl-1*H*-indole-2,3-dione 3-[*N*-(3-bromophenyl) thiosemicarbazone] (4h)

Orange powder (79%): mp 226–229 °C; IR(KBr): υ 3304, 3217 (NH), 1682 (C=O), 1173 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 2.34 (s, 3H, 5-CH_3), 3.21 (s, 3H, N-CH_3), 7.06 (d, *J* = 8.01 Hz, 1H, indole C_6-H), 7.29 (dd, *J* = 7.56, 0.84 Hz, 1H, indole C_7-H), 7.40 (t, *J* = 8.00 Hz, 1H, phenyl C_5-H), 7.48 (d, *J* = 7.68 Hz, 1H, phenyl C_6-H), 7.64 (s, 1H, indole C_4-H), 7.71 (d, *J* = 7.51 Hz, 1H, phenyl C_4-H), 7.91 (t, *J* = 1.90 Hz, 1H, phenyl C_2-H), 10.89 (s, 1H, N_4-H), 12.77 (s, 1H, N_2-H); ^{13}C NMR (HSQC-2D, DMSO-d_6/125 MHz): δ 21.09 (5-CH_3), 26.22 (N-CH_3), 110.13 (indole C_6), 119.53 (indole C_{3a}), 121.22 (phenyl C_3), 121.98 (indole C_4), 124.89 (phenyl C_4), 128.31 (phenyl C_2), 129.16 (phenyl C_6), 130.70 (phenyl C_5), 132.25 (indole C_7), 132.42 (indole C_5), 132.53 (indole C_{7a}), 140.48 (phenyl C_1), 142.15 (indole C_3), 161.29 (indole C_2), 176.69 (C=S); LCMS-APCI (+) *m/z* (%): 403, 405 [(MH^+, MH^++2) 36, 33], 401 (100). Anal. Calcd for $C_{17}H_{15}$ BrN_4OS (403.29): C, 50.63; H, 3.75; N, 13.89. Found: C, 51.12; H, 3.57; N, 13.95.

4.4.9. 1,5-Dimethyl-1*H*-indole-2,3-dione 3-[*N*-(4-nitrophenyl) thiosemicarbazone] (4i)

Orange powder (66%): mp 255–256 °C; IR(KBr): υ 3285 (NH), 1672 (C=O), 1168 (C=S). ^{1}H NMR (DMSO-d_6/500 MHz): δ 2.33 (s, 3H, 5-CH_3), 3.20 (s, 3H, N-CH_3), 7.04 (d, *J* = 7.81 Hz, 1H indole C_6-H), 7.28 (d, *J* = 7.80 Hz, 1H, indole C_7-H), 7.63 (s, 1H, phenyl C_4-H), 8.07 (d, *J* = 8.78 Hz, 2H, phenyl $C_{2,6}$-H), 8.28 (d, *J* = 8.78 Hz, 2H, phenyl $C_{3,5}$-H), 11.17 (s, 1H, N_4-H), 12.91 (s, 1H, N_2-H). Anal. Calcd for $C_{17}H_{15}N_5O_3S$ (369.39): C, 55.27; H, 4.09; N, 18.96. Found: C, 54.66; H, 3.69; N, 18.81.

4.4.10. 1-Methyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-(*N*-methylthiosemicarbazone) (4j)

Yellow powder (82%): mp 218–20 °C; IR(KBr): υ 3355, 3279 (NH), 1689 (C=O), 1124 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 3.09 (d, *J* = 4.60 Hz, 3H, $NHCH_3$), 3.23 (s, 3H, N-CH_3), 7.25 (d, *J* = 8.50 Hz, 1H, indole C_7-H), 7.46 (dd, *J* = 8.50, 1.70 Hz, 1H, indole C_6-H), 7.66 (d, *J* = 1.30 Hz, 1H, indole C_4-H), 9.41 (q, *J* = 4.50 Hz, 1H, N_4-H), 12.38 (s, 1H, N_2-H); ^{13}C NMR APT (DMSO-d_6,/125 MHz): δ 26.35 (N-CH_3), 31.81 ($NHCH_3$), 111.52 (indole C_7), 113.92 (indole C_4), 120.67 (q, *J* = 255.85, CF_3O), 121.28 (indole C_{3a}), 124.24 (indole C_6), 130.08 (indole C_{7a}), 142.78 (indole C_3), 144.41 (indole C_5), 161.30 (indole C_2), 178.05 (C=S). Anal. Calcd for $C_{12}H_{11}F_3N_4O_2S$ (332.30): C, 43.37; H, 3.34; N, 16.86. Found: C, 44.09; H, 3.00; N, 17.08.

4.4.11. 1-Methyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-(*N*-ethylthiosemicarbazone) (4k)

Yellow powder (81%): mp 217–219 °C; IR(KBr): υ 3372, 3320 (NH), 1685 (C=O), 1176 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 1.11 (t, *J* = 7.1 Hz, 3H, ethyl CH_3), 3.14 (s, 3H, N-CH_3), 3.56 (p, *J* = 6.20 Hz, 2H, ethyl CH_2), 7.16 (d, *J* = 8.50 Hz, 1H, indole C_7-H), 7.37 (dd, *J* = 8.50, 1.70 Hz, 1H, indole C_6-H), 7.60 (d, *J* = 1.40 Hz, 1H, indole C_4-H), 9.34 (t, *J* = 5.70 Hz, 1H, N_4-H), 12.27 (s, 1H, N_2-H); LCMS-APCI (- / +) *m/z* (%): 347 (MH^+, 100), 345 (MH^-, 100). Anal. Calcd for $C_{13}H_{13}F_3N_4O_2S$ (346.32): C, 45.08; H, 3.78; N, 16.18. Found: C, 45.85; H, 3.72; N, 16.47.

4.4.12. 1-Methyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-(*N*-allylthiosemicarbazone) (4l)

Yellow crystals (79%): mp 181–182 °C; IR(KBr): υ 3344, 3210 (NH), 1682 (C=O), 1155 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 3.29 (s, 3H, N-CH_3), 4.26 (t, *J* = 5.86 Hz, 2H, allyl C_1-H), 5.14 (dd, *J* = 10.25, 1.46 Hz, 1H, allyl C_3-H_{cis}), 5.20 (dd, *J* = 17.09, 1.46 Hz, 1H, allyl C_3-H_{trans}), 5.88–5.96 (m, 1H, allyl C_2-H), 7.23 (d, *J* = 8.78 Hz, 1H, indole C_7-H), 7.44 (dd, *J* = 7.52, 1.95 Hz, 1H, indole C_6-H), 7.70 (s, 1H, indole C_4-H), 9.56 (t, *J* = 5.85 Hz, 1H, N_4-H), 12.41 (s, 1H, N_2-H). Anal. Calcd for $C_{14}H_{13}F_3N_4O_2S$ (358.33): C, 46.92; H, 3.66; N, 15.64. Found: C, 46.71; H, 3.77; N, 15.61.

4.4.13. 1-Methyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-(*N*-butylthiosemicarbazone) (4m)

Yellow powder (76%): mp 179 °C; IR(KBr): υ 3364, 3336 (NH), 1685 (C=O), 1168 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 0.93 (t, *J* = 7.30 Hz, 3H, butyl CH_3), 1.32–1.38 (m, 2H, butyl C_3-H_2), 1.63 (p, *J* = 7.40 Hz, 2H, butyl C_2-H_2), 3.23 (s, 1H, N-CH_3), 3.61 (q, *J* = 6.90 Hz, 2H, butyl C_1-H_2), 7.18 (d, *J* = 8.50 Hz, 1H, indole C_7-H), 7.39 (dd, *J* = 8.50, 1.60 Hz, 1H, indole C_6-H), 7.65 (d, *J* = 1.60 Hz, 1H, indole C_4-H), 9.34 (t, *J* = 5.90 Hz, 1H, N_4-H), 12.31 (s, 1H, N_2-H); ^{13}C NMR (HETCOR-2D, DMSO-d_6/125 MHz): δ 14.16 (CH_3), 20.07 (butyl C_3), 26.23 (N-CH_3), 30.96 (butyl C_2), 44.43 (butyl C_1), 111.27 (indole C_7), 114.09 (indole C_4), 120.66 (q, *J* = 255.70, CF_3O), 121.19 (indole C_{3a}), 124.05 (indole C_6), 129.88 (indole C_{7a}), 142.59 (indole C_3), 144.41 (indole C_5), 161.16 (indole C_2), 177.29 (C=S). Anal. Calcd for $C_{15}H_{17}F_3N_4O_2S$ (374.38): C, 48.12; H, 4.58; N, 14.97. Found: C, 48.37; H, 4.50; N, 15.03.

4.4.14. 1-Methyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-(*N*-cyclohexylthiosemicarbazone) (4n)

Yellow powder (78%): mp 228–229 °C; IR(KBr): υ 3364, 3319 (NH), 1684 (C=O), 1167 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 1.29–1.94 (m, 10H, cyclohexyl $C_{2,3,4,5,6}$-H), 3.23 (s, 3H, N-CH_3),

4.09–4.32 (m, 1H, cyclohexyl C_1-H), 7.25 (d, J = 8.60 Hz, 1H, indole C_7-H), 7.45, 7.48 (2xdd, J = 8.50, 2.30 Hz, 1H, indole C_6-H), 7.79 (d, J = 2.10 Hz, 1H, indole C_4-H), 8.93 (d, J = 8.50 Hz, 1H, N_4-H), 12.43 (s, 1H, N_2-H); LCMS-APCI (+) m/z (%): 401 (MH^+, 100). Anal. Calcd for $C_{17}H_{19}F_3N_4O_2S$ (400.41): C, 50.99; H, 4.78; N, 13.99. Found: C, 51.41; H, 4.85; N, 14.05.

4.4.15. 1-Methyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-(*N*-benzylthiosemicarbazone) (4o)

Yellow powder (79%): mp 200–201 °C; IR(KBr): υ 3349, 3211 (NH), 1685 (C=O), 1167 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 3.22 (s, 3H, N-CH_3), 4.89 (d, J = 6.35 Hz, 2H, benzyl CH_2), 7.23 (d, J = 8.30 Hz, 1H, indol C_7-H), 7.25–7.27 (m, 1H, benzyl C_4-H), 7.32–7.34 (m, 4H, benzyl $C_{2,3,5,6}$-H), 7.44 (dd, J = 8.78, 1.95 Hz, 1H, indole C_6-H), 7.67 (d, J = 1.95 Hz, 1H, indole C_4-H), 9.92 (t, J = 6.34 Hz, 1H, N_4-H), 12.47 (s, 1H, N_2-H). Anal. Calcd for $C_{18}H_{15}F_3N_4O_2S$ (408.39): C, 52.94; H, 3.70; N, 13.72. Found: C, 52.82; H, 3.66; N, 13.65.

4.4.16. 1-Methyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-(*N*-phenylthiosemicarbazone) (4p)

Orange powder (75%): mp 203–205 °C; IR(KBr): υ 3322, 3226 (NH), 1686 (C=O), 1157 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 3.23 (s, 3H, N-CH_3), 7.23 (d, 1H, indol C_7-H), 7.29 (t, J = 7.32 Hz, 1H, phenyl C_4-H), 7.43 (t, 3H, phenyl $C_{3,5}$-H, indole C_6-H), 7.58 (d, J = 7.80 Hz, 1H, phenyl $C_{2,6}$-H), 7.81 (s, 1H, indole C_4-H), 10.88 (s, 1H, N_4-H), 12.56 (s, 1H, N_2-H); ^{13}C NMR (HSQC-2D, DMSO-d_6/125MHz): δ 26.61 (N-CH_3), 111.71 (indole C_7), 114.00 (indole C_4), 119.89 (OCF_3), 121.42 (indole C_{3a}), 124.67 (indole C_6), 126.51 (phenyl $C_{2,6}$), 127.04 (phenyl C_4), 129.19 (phenyl $C_{3,5}$), 130.96 (indole C_{7a}), 138.97 (phenyl C_1), 143.20 (indole C_3), 144.75 (indole C_5), 161.59 (indole C_2), 177.11 (C=S); LCMS-APCI (-) m/z (%): 393 (MH^-, 100). Anal. Calcd for $C_{17}H_{13}F_3N_4O_2S$ (394.37): C, 51.77; H, 3.32; N, 14.21. Found: C, 51.84; H, 3.08; N, 14.32.

4.4.17. 1-Methyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-[*N*-(4-methylphenyl)thiosemicarbazone] (4q)

Orange crystals (74%): mp 216–217 °C; IR(KBr): υ 3322, 3232 (NH), 1686 (C=O), 1149 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 2.34 (s, 3H, CH_3), 3.26 (s, 3H, N-CH_3), 7.24–7.48 (m, 6H, indole C_7-H, C_6-H, C_6H_4), 7.85 (d, J = 1.20 Hz, 1H, indol C_4-H), 10.87 (s, 1H, N_4-H), 12.57 (s, 1H, N_2-H). Anal. Calcd for $C_{18}H_{15}F_3N_4O_2S$ (408.39): C, 52.94; H, 3.70; N, 13.72. Found: C, 53.00; H, 3.67; N, 13.86.

4.4.18. 1-Methyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-[*N*-(4-fluorophenyl) thiosemicarbazone] (4r)

Yellow powder (77%): mp 214–215 °C; IR(KBr): υ 3314, 3250 (NH), 1687 (C=O), 1151 (C=S).); ^{1}H NMR (DMSO-d_6/500 MHz): δ 3.24 (s, 3H, N-CH_3), 7.25 (d, J = 8.79 Hz, 1H, indole C_7-H), 7.26 (t, 2H, J = 8.78 Hz, phenyl $C_{3,5}$-H), 7.46 (d, J = 8.05 Hz, 1H, indole C_6-H), 7.58 (dd, 2H, J = 8.78, 1.95 Hz, phenyl $C_{2,6}$-H), 7.80 (s, 1H, indole C_4-H), 10.89 (s, 1H, N_4-H), 12.58 (s, 1H, N_2-H). Anal. Calcd for $C_{17}H_{12}F_4N_4O_2S$ (412.36): C, 49.52; H, 2.93; N, 13.59. Found: C, 49.36; H, 2.85; N, 13.61.

4.4.19. 1-Methyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-[*N*-(4-chlorophenyl) thiosemicarbazone] (4s)

Yellow powder (89%): mp 221–222 °C; IR(KBr): υ 3353, 3200 (NH), 1678 (C=O), 1168 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 3.26 (s, 3H, N-CH_3), 7.28 (d, J = 8.60 Hz, 1H, indol C_7-H), 7.49–7.53 (m, 3H, indol C_6-H, phenyl $C_{3,5}$-H), 7.66 (d, J = 8.70 Hz, 2H, phenyl $C_{2,6}$-H), 7.83 (d, J = 1.50 Hz, 1H, indol C_4-H), 10.95 (s, 1H, N_4-H), 12.64 (s, 1H, N_2-H); ^{13}C NMR (APT, DMSO-d_6/125 MHz): δ 26.62 (N-CH_3), 111.77 (indole C_7), 114.89 (indole C_4), 121.33 (OCF_3), 121.92 (indole C_{3a}), 124.80 (indole C_6), 128.11 (phenyl $C_{2,6}$), 129.96 (phenyl $C_{3,5}$), 131.06 (phenyl C_4), 131.24 (indole C_{7a}), 137.94 (phenyl C_1), 143.28 (indole C_3), 144.74 (indole C_5), 161.59 (indole C_2), 177.15 (C=S); LCMS-APCI (−/+) m/z (%): 429 (MH^+, 26), 79 (100), 427 (MH^-, 100). Anal. Calcd for $C_{17}H_{12}ClF_3N_4O_2S.½\ H_2O$ (437.82): C, 46.63; H, 2.99; N, 12.79. Found: C, 46.68; H, 2.76; N, 13.16.

4.4.20. 1-Methyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-[*N*-(4-bromophenyl) thiosemicarbazone] (4t)

Yellow powder (77%): mp 223 °C; IR(KBr): υ 3317, 3230 (NH), 1685 (C=O), 1150 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 3.23 (s, 3H, N-CH_3), 7.23 (d, J = 8.79 Hz, 1H, indole C_7-H), 7.45 (d, J = 7.81 Hz, 1H, indole C_6-H), 7.60 (q, J = 8.78 Hz, 4H, phenyl $C_{3,4,5,6}$-H), 7.79 (s, 1H, indole C_4-H), 10.89 (s, 1H, N_4-H), 12.61 (s, 1H, N_2-H). Anal. Calcd for $C_{17}H_{12}BrF_3N_4O_2S$ (473.26): C, 43.14; H, 2.56; N, 11.84. Found: C, 42.69; H, 2.36; N, 11.78.

4.4.21. 1-Methyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-[*N*-(4-nitrophenyl) thiosemicarbazone] (4y)

Yellow powder (94%): mp 249 °C; IR(KBr): υ 3335, 3290 (NH), 1679 (C=O), 1163 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ 3.24 (s, 3H, N-CH_3), 7.26 (d, J = 8.30 Hz, 1H, indole C_7-H), 7.48 (dd, J = 8.54, 1.46 Hz, 1H, indole C_6-H), 7.81 (d, J = 1.47 Hz, 1H, indole C_4-H), 8.06 (dd, J = 6.83, 1.95 Hz, 2H, phenyl $C_{2,6}$-H), 8.29 (dd, J = 7.32, 1.95 Hz, 2H, phenyl $C_{3,5}$-H), 11.14 (s, 1H, N_4-H), 12.78 (s, 1H, N_2-H). Anal. Calcd for $C_{17}H_{12}F_3N_5O_4S$ (439.36): C, 46.47; H, 2.75; N, 15.94. Found: C, 46.17; H, 2.70; N, 16.75.

4.5. General method for the synthesis of 5-trifluoromethoxy-1-morpholinomethyl-1*H*-indole-2,3-dione 3-thiosemicarbazones (5a–m)

To a suspension of **3h–t** (2 mmol) in absolute ethanol (20 mL), 37% formaldehyde solution (0.5 mL) and morpholine (2 mmol) were added dropwise with vigorous stirring. After combining all reagents, the reaction mixture was stirred at room temperature for 10 h. The solid product was filtered and washed with petroleum ether.

4.5.1. 5-Trifluoromethoxy-1-(morpholin-4-ylmethyl)-1*H*-indole-2,3-dione 3-(*N*-methylthiosemicarbazone) (5a)

Yellow powder (82%): mp 201–202 °C; IR (KBr): υ 3305, 3239 (NH), 1694 (C=O), 1155 (C=S); ^{1}H NMR (DMSO/d_6, 400 MHz): δ 2.56 (br t, J = 4.00 Hz, 4H, morph. $C_{3,5}$-H), 3.10 (d, J = 4.57 Hz, 3H, CH_3), 3.54 (br t, J = 4.30 Hz, 4H, morph. $C_{2,6}$-H), 4.51 (s, 2H, N-CH_2-N), 7.38 (d, J = 8.60 Hz, 1H, indole C_7-H), 7.44 (dd, J = 8.60, 1.80 Hz, 1H, indole C_6-H), 7.69 (d, J = 1.20 Hz, 1H, indole C_4-H), 9.43 (q, J = 4.50 Hz, 1H, N_4-H), 12.37 (s, 1H, N_2-H); ^{13}C NMR (APT, DMSO/d_6, 125 MHz): δ 31.78 ($NHCH_3$), 50.93 (morph. $C_{3,5}$), 61.89 (morph. $C_{2,6}$), 66.44 (N-CH_2-N), 112.77 (indole C_7), 113.83 (indole C_4), 121.37 (indole C_{3a}), 121.93 (q, J = 256.10, CF_3O), 124.04 (indole C_6), 129.77 (indole C_{7a}), 142.56 (indole C_3), 144.50 (indole C_5), 162.06 (indole C_2), 178.00 (C=S). Anal. Calcd for $C_{16}H_{18}F_3N_5O_3S$ (417.40): C, 46.04; H, 4.35; N, 16.78. Found: C, 45.81; H, 4.41; N, 16.74.

4.5.2. 5-Trifluoromethoxy-1-(morpholin-4-ylmethyl)-1*H*-indole-2,3-dione 3-(*N*-ethylthiosemicarbazone) (5b)

Yellow powder (78%): mp 205–207 °C; IR (KBr): υ 3260 (NH), 1695 (C=O), 1165 (C=S); ^{1}H NMR (DMSO/d_6, 400 MHz): δ 1.11 (t, J = 7.10 Hz, 3H, ethyl CH_3), 2.47 (br t, J = 4.30 Hz, 4H, morph. $C_{3,5}$-H), 3.45 (br t, J = 4.40 Hz, 4H, morph. $C_{2,6}$-H), 3.56 (p, J = 6.20 Hz, 2H, ethyl CH_2), 4.41 (s, 2H, N–CH_2–N), 7.28 (d, J = 8.60 Hz, 1H, indole C_7-H), 7.35 (dd, J = 8.60, 1.80 Hz, 1H, indole C_6-H), 7.62 (d, J = 1.40 Hz, 1H, indole C_4-H), 9.35 (t, J = 5.80 Hz, 1H, N_4-H), 12.25 (s, 1H, N_2-H); LCMS-APCI (-/+): m/z (%) 432 (MH^+, 14), 333 (100), 265 (99), 430 (MH^-, 8), 265 (100). Anal. Calcd for $C_{17}H_{20}F_3N_5O_3S$

(431.43): C, 47.33; H, 4.67; N, 16.23. Found: C, 47.48; H, 4.35; N, 16.27.

4.5.3. 5-Trifluoromethoxy-1-(morpholin-4-ylmethyl)-1*H*-indole-2,3-dione 3-(*N*-allylthiosemicarbazone) (5c)

Dark yellow powder (84%): mp 153 °C; IR (KBr): υ 3250 (NH), 1694 (C=O), 1162 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ *2.55* (t, *J* = 4.27 Hz, 4H, morph. $C_{3,5}$-H), 3.53 (t, *J* = 4.42 Hz, 4H, morph. $C_{2,6}$-H), 4.26 (t, *J* = 5.64 Hz, 2H, allyl C_1-H), 4.49 (s, 2H, N-CH_2-N), 5.15 (dd, *J* = 10.07, 1.52 Hz, 1H, allyl C_3-H_{cis}), 5.20 (dd, *J* = 17.38, 1.52 Hz, 1H, allyl C_3-H_{trans}), 5.87–5.95 (m, 1H, allyl C_2-H), 7.36 (d, *J* = 8.54 Hz, 1H, indole C_7-H), 7.42 (dd, *J* = 8.54, 1.83 Hz, 1H, indole C_6-H), 7.72 (d, *J* = 1.52 Hz, 1H, indole C_4-H), 9.59 (t, *J* = 5.95 Hz, 1H, N_4-H), 12.38 (s, 1H, N_2-H). Anal. Calcd for $C_{18}H_{20}F_3N_5O_3S·½H_2O$ (452.45): C, 47.78; H, 4.67; N, 15.47. Found: C, 48.20; H, 5.05; N, 15.49.

4.5.4. 5-Trifluoromethoxy-1-(morpholin-4-ylmethyl)-1*H*-indole-2,3-dione 3-(*N*-butylthiosemicarbazone) (5d)

Yellow powder (78%): mp 129–132 °C; IR (KBr): υ 3302 (NH), 1692 (C=O), 1162 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ 0.93 (t, *J* = 7.30 Hz, 3H, butyl C_4-H), 1.32–1.39 (m, 2H, butyl C_3-H), 1.63 (p, *J* = 7.40 Hz, 2H, butyl C_2-H), 2.56 (t, *J* = 3.80 Hz, 4H, morph. $C_{3,5}$-H), 3.54 (t, *J* = 4.20 Hz, 4H, morph. $C_{2,6}$-H), 3.63 (q, *J* = 6.80 Hz, 2H, butyl C_1-H), 4.50 (s, 2H, N–CH_2–N), 7.37 (d, *J* = 8.60, Hz, 1H, indole C_7-H), 7.42 (dd, *J* = 8.60, 1.80 Hz, 1H, indole C_6-H), 7.72 (d, *J* = 1.60 Hz, 1H, indole C_4-H), 9.42 (t, *J* = 5.90 Hz, 1H, N_4-H), 12.34 (s, 1H, N_2-H); ^{13}C NMR (HETCOR-2D, DMSO/d_6, 100 MHz): 14.19 (butyl C_4), 20.05 (butyl C_3), 30.97 (butyl C_2), 44.43 (butyl C_1), 50.93 (morph. $C_{3,5}$), 61.86 (morph. $C_{2,6}$), 66.44 (N–CH_2–N), 112.79 (indole C_7), 114.06 (indole C_4), 120.67 (q, *J* = 258.10, CF_3O), 121.39 (indole C_{3a}), 124.08 (indole C_6), 129.90 (indole C_{7a}), 142.63 (indole C_3), 144.48 (indole C_5), 162.10 (indole C_2), 177.28 (C=S). Anal. Calcd for $C_{19}H_{24}F_3N_5O_3S$ (459.48): C, 49.66; H, 5.26; N, 15.24. Found: C, 49.44; H, 5.08; N, 15.01.

4.5.5. 5-Trifluoromethoxy-1-(morpholin-4-ylmethyl)-1*H*-indole-2,3-dione 3-(*N*-cyclohexylthiosemicarbazone) (5e)

Orange crystals (93%): mp 178–180 °C; IR (KBr): υ 3281 (NH), 1696 (C=O), 1160 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ *1.*03–1.86 (m, 10H, cycl. $C_{2,3,4,5,6}$-H), 2.46 (br t, *J* = 4.30 Hz, morph. $C_{3,5}$-H), 3.45 (br t, *J* = 4.40 Hz, 4H, morph. $C_{2,6}$-H), 4.10–4.13 (m, 1H, cycl. C_1-H), 4.41 (s, 2H, N–CH_2–N), 7.28 (d, *J* = 8.60 Hz, 1H, indole C_7-H), 7.36 (dd, *J* = 8.70, 1.5 Hz, 1H, indole C_6-H), 7.72 (d, *J* = 2.10 Hz, 1H, indole C_4-H), 8.86 (d, *J* = 8.40 Hz, 1 H, N_4-H), 12.29 (s, 1H, N_2-H); LCMS-APCI (−/+): *m/z* (%) 486 (MH^+, 1), 387 (100), 484 (MH^-, 5), 420 (100). Anal. Calcd for $C_{21}H_{26}F_3N_5O_3S·½H_2O$ (494.53): C, 51.00; H, 5.50; N, 14.16. Found: C, 50.65; H, 5.57; N, 14.00.

4.5.6. 5-Trifluoromethoxy-1-(morpholin-4-ylmethyl)-1*H*-indole-2,3-dione 3-(*N*-benzylthiosemicarbazone) (5f)

Yellow powder (91%): mp 168–169 °C; IR (KBr): υ 3270 (NH), 1694 (C=O), 1153 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ *2.55* (t, *J* = 4.27 Hz, 4H, morph. $C_{3,5}$-H), 3.53 (t, *J* = 4.27 Hz, 4H, morph. $C_{2,6}$-H), 4.49 (s, 2H, N–CH_2–N), 4.89 (d, *J* = 6.10 Hz, 2H, benzyl CH_2), 7.25 (t, *J* = 6.41 Hz, 1H, benzyl C_4-H), 7.32–7.36 (m, 5H, indole C_7-H, benzyl $C_{2,3,5,6}$-H), 7.42 (dd, *J* = 8.84, 1.83 Hz, 1H, indole C_6-H), 7.69 (br s, 1H, indole C_4-H), 9.95 (t, *J* = 6.25 Hz, 1H, N_4-H), 12.44 (s, 1H, N_2-H). Anal. Calcd for $C_{22}H_{22}F_3N_5O_3S$ (493.50): C, 53.54; H, 4.49; N, 14.19. Found: C, 53.79; H, 4.30; N, 14.31.

4.5.7. 5-Trifluoromethoxy-1-(morpholin-4-ylmethyl)-1*H*-indole-2,3-dione 3-(*N*-phenylthiosemicarbazone) (5g)

Yellow powder (68%): mp 182–183 °C; IR (KBr): υ 3227 (NH), 1697 (C=O), 1159 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ *2.57* (t, *J* = 4.27 Hz, 4H, morph. $C_{3,5}$-H), 3.54 (t, *J* = 4.27 Hz, 4H, morph. $C_{2,6}$-H), 4.51 (s, 2H, N–CH_2–N), 7.29 (t, *J* = 7.32 Hz, 1H, phenyl C_4-H), 7.37 (d, *J* = 8.55 Hz, 1H, indole C_7-H), 7.43 (d, *J* = 8.23 Hz, 1H, indole C_6-H), 7.44 (d, *J* = 7.63 Hz, 2H, phenyl $C_{3,5}$-H), 7.58 (d, *J* = 7.62 Hz, 2H, phenyl $C_{2,6}$-H), 7.85 (br s, 1H, indole C_4-H), 10.91 (s, 1H, N_4-H), 12.55 (s, 1H, N_2-H). Anal. Calcd for $C_{20}H_{20}F_3N_5O_3S·½H_2O$ (488.48): C, 51.63; H, 4.33; N, 14.33. Found: C, 51.73; H, 4.25; N, 14.54.

4.5.8. 5-Trifluoromethoxy-1-(morpholin-1-ylmethyl)-1*H*-indole-2,3-dione 3-[*N*-(4-methylphenyl) thiosemicarbazone) (5h)

Dark yellow crystals (71%): mp 169°C; IR (KBr): υ 3303, 3231 (NH), 1697 (C=O), 1156 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ *2.*34 (s, 3H, CH_3), 2.59 (br t, *J* = 4.20 Hz, 4H, morph. $C_{3,5}$-H), 3.56 (br t, *J* = 4.30 Hz, 4H, morph. $C_{2,6}$-H), 4.53 (s, 2H, N–CH_2–N), 7.25 (d, *J* = 8.30 Hz, 2H, phenyl $C_{3,5}$-H), 7.40 (d, *J* = 8.60 Hz, 1H, indole C_7-H), 7.46 (d, *J* = 8.30 Hz, 1 H, indole C_6-H, phenyl $C_{2,6}$-H), 7.87 (br s, 1H, indole C_4-H), 10.88 (s, 1H, N_4-H), 12.54 (s, 1H, N_2-H); ^{13}C NMR (APT DMSO-d_6/125 MHz): δ 21.32 (CH_3), 51.20 (morph. C_3, C_5), 62.19 (N–CH_2–N), 66.69 (morph. C_2, C_6), 113.10 (indole C_7), 114.77 (indole C_4), 120.91 (q, *J* = 255.93 Hz, CF_3O), 121.59 (indole C_{3a}), 124.53 (indole C_6), 126.38 (phenyl C_3, C_5), 129.63 (phenyl C_2, C_6),130.69 (indole C_{7a}), 136.35 (phenyl C_4), 136.41 (phenyl C_1), 143.08 (indole C_3), 144.79 (indole C_5), 162.43 (indole C_2), 177.06 (C=S); LCMS-APCI (+): *m/z* (%) 494 (MH^+, 2), 439 (100). Anal. Calcd for $C_{22}H_{22}F_3N_5O_3S$ (493.50): C, 53.54; H, 4.49; N, 14.19. Found: C, 53.88; H, 4.36; N, 14.19.

4.5.9. 5-Trifluoromethoxy-1-(morpholin-1-ylmethyl)-1*H*-indole-2,3-dione 3-[*N*-(4-methoxyphenyl) thiosemicarbazone) (5i)

Orange powder (90%): mp 171–173 °C; IR (KBr): υ 3316, 3210 (NH), 1689 (C=O), 1154 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ *2.*57 (t, *J* = 4.39 Hz, 4H, morph. $C_{3,5}$-H), 3.54 (t, *J* = 4.39 Hz, 4H, morph. $C_{2,6}$-H), 3.77 (s, 3H, OCH_3), 4.51 (s, 2H, N–CH_2–N), 6.98 (d, *J* = 8.79 Hz, 2H, phenyl $C_{3,5}$-H), 7.37 (d, *J* = 8.30 Hz, 1H, indole C_7-H), 7.42–7.45 (m, 3H, indole C_6-H, phenyl $C_{2,6}$-H), 7.84 (br s, 1H, indole C_4-H), 10.83 (s, 1H, N_4-H), 12.51 (s, 1H, N_2-H). Anal. Calcd for $C_{22}H_{22}F_3N_5O_4S$ (509.50): C, 51.86; H, 4.35; N, 13.75. Found: C, 51.92; H, 4.04; N, 13.78.

4.5.10. 5-Trifluoromethoxy-1-(morpholin-4-ylmethyl)-1*H*-indole-2,3-dione 3-[*N*-(4-fluorophenyl) thiosemicarbazone) (5j)

Orange powder (76%): mp 162–163 °C; IR (KBr): υ 3289, 3215 (NH), 1703 (C=O), 1110 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ *2.*57 (t, *J* = 4.27 Hz, 4H, morph. $C_{3,5}$-H), 3.54 (t, *J* = 4.27 Hz, 4H, morph. $C_{2,6}$-H), 4.51 (s, 2H, N–CH_2–N), 7.27 (t, *J* = 8.84 Hz, 2H, phenyl $C_{3,5}$-H), 7.38 (d, *J* = 8.55 Hz, 1H, indole C_7-H), 7.44 (dd, *J* = 8.54, 2.13 Hz, 1H, indole C_6-H), 7.58 (dd, *J* = 8.85, 4.88 Hz, 2H, phenyl $C_{2,6}$-H), 7.82 (br s, indole C_4-H), 10.91 (s, 1H, N_4-H), 12.56 (s, 1H, N_2-H). Anal. Calcd for $C_{21}H_{19}F_4N_5O_3S$ (497.46): C, 50.70; H, 3.85; N, 14.08. Found: C, 50.06; H, 3.53; N, 13.82.

4.5.11. 5-Trifluoromethoxy-1-(morpholin-4-ylmethyl)-1*H*-indole-2,3-dione 3-[*N*-(4-chlorophenyl) thiosemicarbazone) (5k)

Yellow powder (75%): mp 180–182 °C; IR (KBr): υ 3305, 3214 (NH), 1698 (C=O), 1164 (C=S); ^{1}H NMR (DMSO-d_6/400 MHz): δ *2.*59 (br t, *J* = 4.20 Hz, 4H, morph. $C_{3,5}$-H), 3.56 (br t, *J* = 4.30 Hz, 4H, morph. $C_{2,6}$-H), 4.53 (s, 2H, N–CH_2–N), 7.40 (d, *J* = 8.60 Hz, 1H, indole C_7-H), 7.48 (dd, *J* = 8.80, 1.50 Hz, 1H, indole C_6-H), 7.51 (d, *J* = 8.70 Hz, 2H, phenyl $C_{3,5}$-H), 7.66 (d, *J* = 8.70 Hz, 2H, phenyl $C_{2,6}$-H), 7.85 (d, *J* = 1.50 Hz, indole C_4-H), 10.97 (s, 1H, N_4-H), 12.61 (s, 1H, N_2-H); LCMS-APCI (−): *m/z* (%) 512 (MH^-, 2), 413, 415 (100, 37). Anal. Calcd for $C_{21}H_{19}ClF_3N_5O_3S$

(513.92): C, 49.08; H, 3.73; N, 13.63. Found: C, 49.12; H, 3.43; N, 13.67.

4.5.12. 5-Trifluoromethoxy-1-(morpholin-4-ylmethyl)-1*H*-indole-2,3-dione 3-[*N*-(4-bromophenyl) thiosemicarbazone) (5l)

Yellow powder (91%): mp 180°C; IR (KBr): υ 3307, 3217 (NH), 1699 (C=O), 1158 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ *2*.57 (t, *J* = 4.39 Hz, 4H, morph. $C_{3,5}$-H), 3.54 (t, *J* = 4.39 Hz, 4H, morph. $C_{2,6}$-H), 4.51 (s, 2H, N–CH_2–N), 7.37 (d, *J* = 8.78 Hz, 1H, indole C_7-H), 7.44 (dd, *J* = 8.78, 1.47 Hz, 1H, indole C_6-H), 7.58 (d, *J* = 8.78 Hz, 2H, phenyl $C_{3,5}$-H), 7.62 (d, *J* = 8.78 Hz, 2H, phenyl $C_{2,6}$-H), 7.83 (d, *J* = 1.46 Hz, indole C_4-H), 10.92 (s, 1H, N_4-H), 12.60 (s, 1H, N_2-H); ^{13}C NMR (HSQC-2D, DMSO-d_6/125 MHz): δ 51.19 (morph. C_3, C_5), 62.22 (N–CH_2–N), 66.69 (morph. C_2, C_6), 113.18 (indole C_7), 114.80 (indole C_4), 119.37 (phenyl C_4), 120.90 (q, *J* = 255.93 Hz, CF_3O), 121.48 (indole C_{3a}), 124.72 (indole C_6), 128.42 (phenyl C_3, C_5), 131.16 (indole C_{7a}), 132.05 (phenyl C_2, C_6), 138.38 (phenyl C_1), 143.20 (indole C_3), 144.79 (indole C_5), 162.44 (indole C_2), 177.07 (C=S). LCMS-APCI (−): *m/z* (%) 556, 558 [(MH^-,MH^-+2) 85, 100]. Anal. Calcd for $C_{21}H_{19}BrF_3N_5O_3S$ (558.37): C, 45.17; H, 3.43; N, 12.54. Found: C, 45.10; H, 3.30; N, 12.98.

4.5.13. 5-Trifluoromethoxy-1-(morpholin-4-ylmethyl)-1*H*-indole-2,3-dione 3-[*N*-(4-nitrophenyl) thiosemicarbazone) (5m)

Dark yellow powder (73%): mp 169–171 °C; IR (KBr): υ 3238 (NH), 1701 (C=O), 1154 (C=S); ^{1}H NMR (DMSO-d_6/500 MHz): δ *2*.58 (t, *J* = 4.27 Hz, 4H, morph. $C_{3,5}$-H), 3.54 (t, *J* = 4.57 Hz, 4H, morph. $C_{2,6}$-H), 4.52 (s, 2H, N–CH_2–N), 7.39 (d, *J* = 8.85 Hz, 1H, indole C_7-H), 7.47 (dd, *J* = 8.84, 1.52 Hz, 1H, indole C_6-H), 7.84 (d, *J* = 1.53 Hz, 1H, indole C_4-H), 8.21 (d, *J* = 9.15 Hz, 2H, phenyl $C_{2,6}$-H), 8.30 (d, *J* = 9.15 Hz, 2H, phenyl $C_{3,5}$-H), 11.15 (s, 1H, N_4-H), 12.76 (s, 1H, N_2-H). Anal. Calcd for $C_{21}H_{19}F_3N_6O_5S$ (524.47): C, 48.09; H, 3.65; N, 16.02. Found: C, 48.08; H, 3.32; N, 15.74.

4.6. In vitro evaluation of antituberculosis activity

The primary screen was conducted against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA).[22] Compounds were tested in 10 twofold dilutions, usually from 100 μg/mL to 0.19 μg/mL. Compounds demonstrating a percent inhibition of bacterial growth of greater than or equal to 90% in the primary screen were retested against *M. tuberculosis* H37Rv to determine the actual minimum inhibitory concentration (MIC) in the MABA. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. This value was determined from the dose-response curve as the IC_{90} using a curve fitting program. Any IC_{90} value of ⩽10 μg/mL was considered "Active" for antitubercular activity. Compounds active in the initial screen were tested for cytotoxicity in VERO cells. After 72 h exposure, viability was assessed using the CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTT) reagent from Promega. Cytotoxicity was determined from the dose-response curve as the IC_{50} using a curve fitting program. Concurrent with the determination of MICs, compounds were tested for IC_{50} in VERO cells at concentrations 10x the MIC for *M. tuberculosis* H37Rv.

4.6.1. Microplate Alamar Blue Assay (MABA)

Antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, Conn.) in order to minimize background fluorescence. Outer perimeter wells were filled with sterile water to prevent dehydration in experimental wells. Initial drug dilutions were prepared in either dimethyl sulfoxide or distilled deionized water, and subsequent twofold dilutions were performed in 0.1 mL of 7H9GC (no Tween 80) in the microplates. BACTEC 12B-passaged inocula were initially diluted 1:2 in 7H9GC, and 0.1 mL was added to wells. The determination of bacterial titer yielded 1×10^6 CFU/mL in plate well for H_{37}Rv. Frozen inocula were initially diluted 1:20 in BACTEC 12B medium followed by a 1:50 dilution in 7H9GC. Addition of 1/10 mL to wells resulted in final bacterial titer of 20×10^5 CFU/mL for H_{37}Rv. Wells containing drug only were used to detect autofluorescence of compounds. Additional control wells consisted of bacteria only (B) and medium only (M). Plates were incubated at 37 °C. Starting at day 4 of incubation, 20 μL of 10 × alamarBlue solution (Alamar Biosciences/Accumed, Westlake, Ohio) and 12.5 μL of 20% Tween 80 were added to one B well and one M well, and plates were reincubated at 37 °C. Wells were observed 12 and 24 h later for a color change from blue to pink and for a reading of ⩾50,000 fluorescence units (FU). Fluorescence was measured in a Cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, Mass.) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. If the B wells become pink after 24 h, the reagent is being added to the entire plate. If the well-remain blue or ⩾50,000 FU is measured, additional M and B wells are tested daily until a color change occurred, at which time reagents are added to all remaining wells. Plates were then incubated at 37 °C, and results were recorded at 24 h post-reagent addition. Visual MICs were defined as the lowest concentration of drug that prevented a color change. For fluorometric MICs, a background subtraction was performed on all wells with a mean of triplicate M wells. Percent inhibition was defined as (1 − (test well FU/mean FU of triplicate B wells) × 100). The lowest drug concentration effecting an inhibition of ⩾90% was considered the MIC.

4.6.2. BACTEC radiometric assay

A total of 1/10 ml of BACTEC 12B-passaged inoculum was delivered without prior dilution into 4 mL of test medium. The determination of bacterial titer yielded average titer of 1×10^5 CFU/ml of BACTEC 12B medium for H_{37}Rv. Frozen inocula were initially diluted 1:20 in BACTEC 12B medium, and then 0.1 mL was delivered to the test medium. This yielded 5.0×10^5 CFU per BACTEC vial for H_{37}Rv. Twofold drug dilutions were prepared in either dimethyl sulfoxide (DMSO) or distilled deionized water and delivered via a 0.5-mL insulin syringe in a 50-μL volume. Drug-free control vials consisted of solvent with bacterial inoculum and solvent with a 1:100 dilution of bacterial inoculum (1:100 controls). Vials were incubated at 37 °C, and the GI was determined in a BACTEC 460 instrument (Becton–Dickinson) until the GI of the 1:100 controls reached at least 30. All vials were read the following day, and the GI and daily change in GI (ΔGI) were recorded for each drug dilution. The MIC was defined as the lowest concentration for which the ΔGI was less than the ΔGI of the 1:100 control. If the GI of the test sample was greater than 100, the sample was scored as resistant even if the ΔGI was less than the ΔGI of the 1:100 control.

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