Synthesis and SAR evaluation of novel thioridazine derivatives active against drug-resistant tuberculosis.

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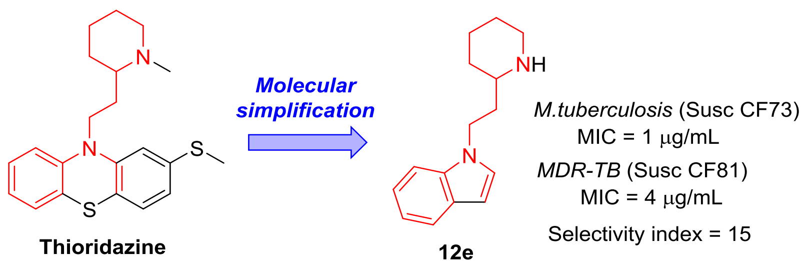
ABSTRACT

The neuroleptic drug thioridazine has been recently repositioned as possible antitubercular drug. Thioridazine showed antitubercular activity against drug resistant mycobacteria but it is endowed with adverse side effects. A small library of thioridazine derivatives has been designed through the replacement of the piperidine and phenothiazine moieties, with the aim to improve the antitubercular activity and to reduce the cytotoxic effects. Among the resulting compounds, the indole derivative **12e** showed an antimycobacterial activity significantly better than thioridazine and a cytotoxicity 15-fold lower.



1. INTRODUCTION

According to the recent World Health Organization annual report, tuberculosis (TB) remains one of the deadliest communicable infections.[1] Nearly one third of the worldwide population is latently infection with *Mycobacterium tuberculosis* (MTB), the etiological agent of tuberculosis in humans, and almost 9 million people develop active TB infections per annum. In addition, 14.8% of global TB patients are co-infected with HIV and can be credited as one of the most common causes of death among AIDS patients.[2,3] This global scenario is due to many causes including the lack of rapid diagnostic tools, the non-compliance of hospitalised patients to the 6-12 months multidrug therapy and institutions lacking the proper drug regimens to treat all the people infected.[4] As a consequence of these transgressions, and after half a century of little to no innovation in the field, MTB have developed multi-drug resistant (MDR),[5-7] extensively-drug resistant (XDR) [8] and totally-drug resistant (TDR) [9] strains, which are resistant to almost all the known available drugs. In 2012, the quinoline derivative bedaquiline [10-11] became the first new drug launched in the market in the last 40 years, since the discovery of rifampin. Currently a number of lead molecules are in clinical trials, such as the diamine SQ109,[12] the fluoroquinolone gatifloxacin [13] and the linezolid.[14] However, the conventional therapeutic approach potentially exacerbates the incidence of new MDR-TB strains and therefore it is inevitable that MTB will evolve resistance against these novel drugs.[15-16]

Conventional drug discovery approaches need the identification of a specific target for the development and optimization of a specific molecule. However, it is well known that singular mutations of the targets active site could result in the nullification of drug activity.[17] The current treatment of TB involves the administration of several drugs simultaneously, this reduces the incidence of resistant MTB strains by avoiding single point mutations resistances against singular treatments. However, several side effects and poor patient compliance are associated with the present multiple therapy. A potentially successful approach to defeat TB is to discover a drug capable of inhibiting multiple MTB targets simultaneously whilst also retaining activity against MDR and latent TB with an ultimate objective of shortening the current TB regimens.

Thioridazine (TZ) **1**, a long established neuroleptic drug, has been recently repositioned as anti-tubercular drug finding application in the treatment of MDR-TB.[18-19] TZ is currently used in therapy as a third line anti-tubercular drug due to the side-effects on the central nervous system and cardiovascular system which restrict its clinical use.[20] Despite the mechanism of action of TZ having not been fully elucidated, recent studies showed that it inhibits efflux pumps in mycobacteria and alters the cell-envelope permeability of MTB.[21-23] Furthermore, TZ **1** is able to affects the physiology of alveolar macrophages, enhancing the retention of potassium ions and promoting the acidification of phagolysosomal vacuole,[24] finally leading to the degradation of intramacrophagic MTB.

Despite the chemistry and structure-activity relationship (SAR) properties of TZ, and related neuroleptic drugs, having been widely investigated in the past, to the best of our knowledge no drug derivatization and optimization studies have been carried out on TZ analogues as inhibitors of MTB.

Herein, we report the synthesis, biological evaluation and SAR studies of a narrow library of novel TZ derivatives. In particular, we aimed at the design and identification of novel TZ analogues with improved activity against TB and MDR-TB strains as well as reduced cytotoxic effects. Three series of derivatives were planned in order to explore the chemical space around the TZ nucleus, as shown in Figure 1. In the first series, the N-methyl substituent on the piperidine ring was removed or replaced with different alkyl groups to evaluate its importance for anti-tubercular activity. In the second series, the piperidine ring of TZ was replaced with different aliphatic heterocyles, keeping fixed the distance between the piperidine nitrogen and the phenothiazine ring.

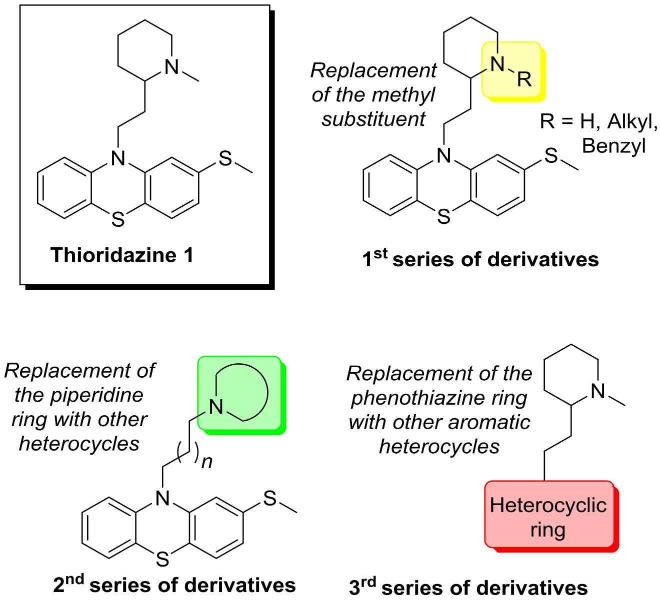
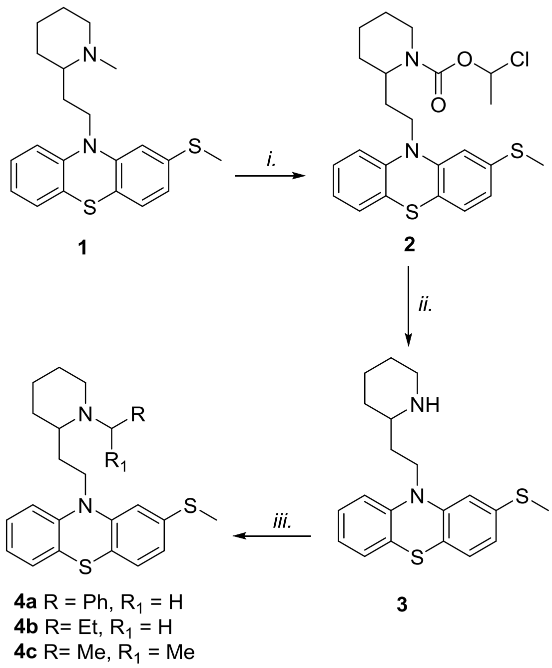


Figure 1. General structures of the thioridazine analogues

The role of the thiomethyl group attached to the phenothiazine ring was also investigated in this series. Finally, in the third series the phenothiazine core, which is responsible for the main side effects on the nervous system, was replaced with different heteroaromatic rings, with the aim to reduce the toxicity of the molecule.

2. RESULTS AND DISCUSSION

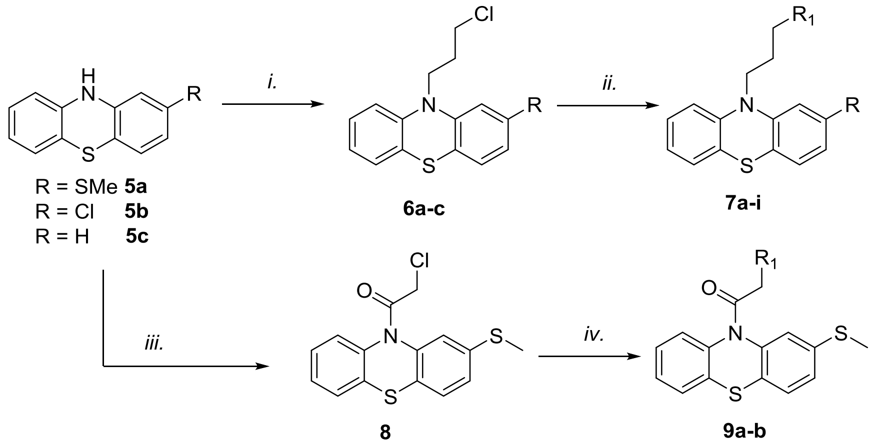
2.1 Chemistry



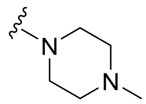
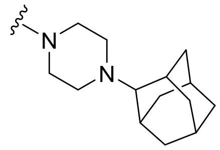
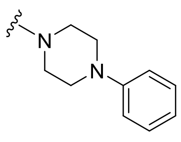
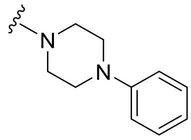
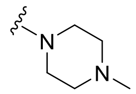
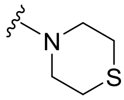
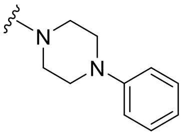
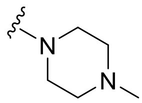
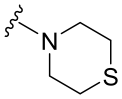
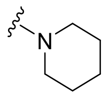
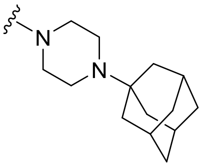
Scheme 1. Synthesis of analogues **4a-c**. Reagents and conditions: *i.* 1-chloro-ethyl-chloroformate, DCE, TEA, reflux, 12 h; *ii.* MeOH, reflux, 12 h; *iii.* NaBH(AcO)$_3$, THF, AcOH, benzaldehyde for **4a**, or propionaldehyde for **4b**, or acetone for **4c**.

A series of *N*-substituted derivatives **4a-c** was first synthesised. TZ was successfully demethylated by treatment with 1-chloro-ethyl-chloroformate in refluxing DCE [25] followed by hydrolysis with MeOH under reflux, leading to derivative **3**. Reductive amination of **3** with different aldehydes/ketones led to the final *N*-alkyl-derivatives **4a-c** in good yields (62-68%). Scheme 1.

Table 1. Synthesis of the compounds **7a-i** and **9a-b**.

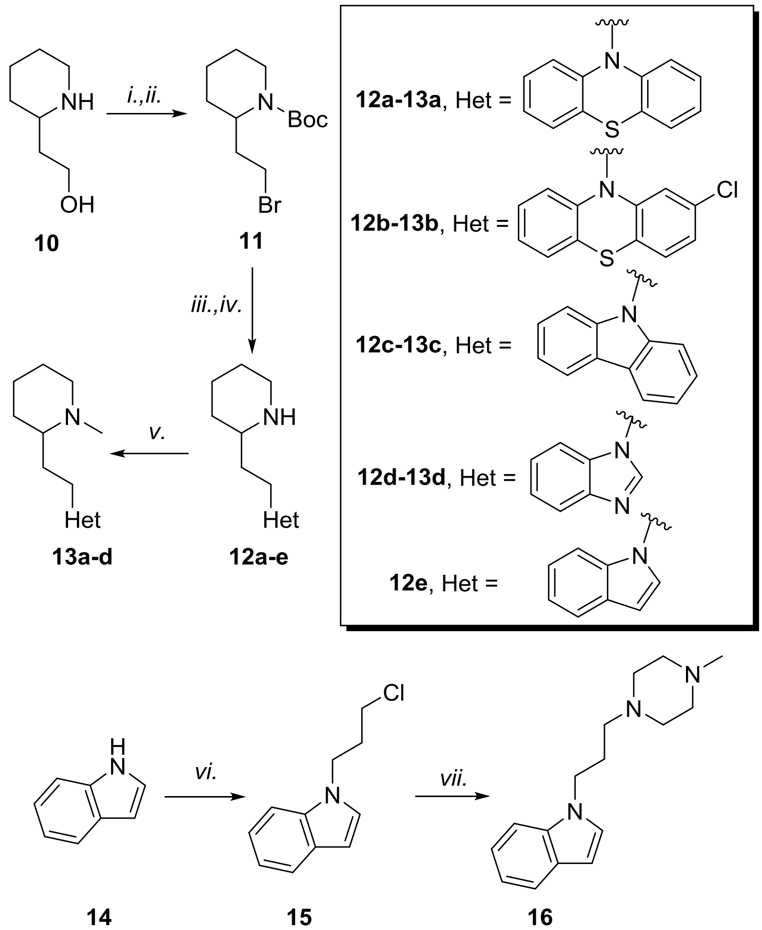


Reagents and conditions: *i.* 1-bromo-3-chloropropane, NaH, DMF, r.t., 12 h; *ii.* amine, DIPEA, NaI, DMF, 150 °C, 3 h; *iii.* 2-chloroacetyl chloride, NaH, DMF, r.t., 12 h; *iv.* amine, NaH, DMF, 150 °C.

Cmpd	R	Amine/R_1	Cmpd	R	
7a	H		**7g**	SMe	
7b	H		**7h**	Cl	
7c	SMe		**7i**	Cl	
7d	SMe		**9a**	SMe	
7e	SMe		**9b**	SMe	
7f	SMe				

A second series of derivatives where the piperidine ring was replaced with different piperazine and thiomorpholine groups was then synthesised (Table 1). In addition, the thio-methyl substituent on the phenothiazine ring was removed or replaced with chlorine, to evaluate its importance for the antitubercular activity.

In particular, the chlorine substituent was chosen on the basis of similarity with chlorpromazine, a phenothiazine derivative closely related to TZ whose efflux pumps inhibitory activity is well known. In detail, the phenothiazines **5a-c** were first reacted with 1-bromo-3-chloropropane to yield the chloroderivatives **6a-c** which were in turn treated with different piperazines and with thiomorpholine to yield the desired products **7a-i**. The thio-methyl-phenothiazine **5a** was also reacted with 2-chloroacetyl chloride leading to **8**, which was in turn converted into derivatives **9a-b** by treatment with methylpiperazine or piperidine.



Scheme 2. Synthesis of analogues **12**, **13** and **16**. Reagents and conditions: *i.* $(Boc)_2O$, $Na_2CO_{3(aq)}$/DCM, r.t., 12 h; *ii.* PPh_3, CBr_4, DCM, r.t., 2 h; *iii.* Heterocycle, NaH, NaI, DMF, r.t., 12 h; *iv.* HCl/EtOAc, r.t., 24 h; *v.* CH_2CO, $NaBH(AcO)_3$, THF, AcOH, r.t., 24 h; *vi.*1-bromo-3-chloropropane, NaH, DMF, r.t., 12 h; *vii.* *N*-methyl-piperazine, DIPEA, NaI, DMF, 150 °C, 3 h.

Finally, the third series of compounds bearing the ethyl-piperidine chain of TZ bound to different aromatic heterocycles was synthesised. Scheme 2. 2-(Piperidin-2-yl)ethanol **10** was converted into the Boc-bromoderivative **11** by treatment with $(Boc)_2O$ followed by reaction with CBr_4. Different heteroaromatic compounds (namely phenothiazine, 2-Cl-phenothiazine, carbazole, indole, and benzimidazole) were then alkylated with **11** and the resulting intermediates were deprotected with TFA yielding the **12a-e** series of compounds. *N*-methylation of **12** through reductive amination led to the methyl derivatives **13a-d**.[26] Finally, **16**, bearing an indole nucleus and a piperazine ring as the aliphatic side chain, were synthesised. Indole **14** was converted into the *N*-chloro-propyl derivative **15**, which yielded the final compound **16** after treatment with *N*-methyl-piperazine.

2.2. Biological evaluation

All the compounds were initially evaluated for their activity against a panel of non-pathogenic mycobacteria strains (namely, *M. smegmatis* mc^{2}155, *M. bovis* BCG and *M. tuberculosis* mc^{2}7000, as shown in Table 2).

Table 2. Activity of TZ derivatives on mycobacterium species (expressed as μg/mL).

Cmpd	*M. smegmatis* mc^2 155	*M. bovis* BCG	*M. tuberculosis*			MDR-TB	
			mc^{2}7000	H37Rv	Susc. (CF73)	CF104	CF81
TZ 1	16	16	8	10	8	11	10
3	16	8	8	100	>100	>100	>100
4a	16	8	8	>100	37	32	44
4b	>64	64	64	45	-	-	-
4c	>64	>64	>64	-	-	-	-
7a	64	64	27	100	>100	>100	>100
7b	>64	>64	>64	-	-	-	-
7c	32	8	27	69	27	23	33
7d	>64	>64	>64	-	-	-	-
7e	>64	>64	>64	-	-	-	-
7f	64	64	64	-	-	-	-
7g	4	>64	>64	43	98	20	48
7h	>64	>64	>64	-	-	-	-
7i	>64	>64	>64	-	-	-	-
9a	>64	>64	>64	-	-	-	-
9b	-	-	-	>100	>100	72	>100

12a	>64	>64	>64	>64	47	>64	58
12b	16	5.3	4	26	11	19	11
12c	>64	>64	>64	-	-	-	-
12d	>64	>64	>64	100	>100	>100	>100
12e	1.6	64	-	2.9	1	10	4
13a	32	64	16	>64	47	>64	58
13b	16	8	8	19	16	16	12
13c	>64	>64	64	60	39	48	46
13d	>64	>64	>64	-	-	-	-
16	>64	>64	>64	-	-	-	-
INH	4	0.063	0.125	0.03	0.03	>25	>25
RIF	-	-	-	0.3	8	>25	>25

A SAR analysis showed that removal of the methyl group of thioridazine did not affect the activity, the desmethyl-thioridazine **3** showing an activity similar to that of **1**. Also the introduction of a benzyl chain as in **4b** maintained a similar activity. On the other hand, the replacement of the piperidine-ethyl moiety with alkyl chains bearing piperazine or thiomorpholine rings as in **7** and **9** led to a dramatic decrease of antimycobacterial activity, with the only exception of the bulky derivative **7g** which showed a MIC = 4 μg/mL on *M. smegmatis*. Moreover, removal of the methylthio substituent of **1** and **3** (as in **13a** and **12a**), as well as replacement of the phenothiazine scaffold with different heterocyclic moieties (as in **13c-d** and **12c-d**) led to a significant loss in activity.

Table 3. Cytotoxicity of compounds **1**, **12b**, **12e**, **13b** expressed as μg/mL and Selectivity Index expressed as absolute number

Cmpds	IC_{50} MRC-5	SI[a]
TZ 1	8.2 μg/mL	1
12b	10 μg/mL	0.9
12e	15 μg/mL	15
13b	13 μg/mL	0.8

[a]Selectivity index is calculated as the ratio between the *M. tuberculosis* Susc. (CF73) MIC and the MRC-5 IC_{50}.

On the contrary, replacement of the same SMe group with a chloride group (as in **12b** and **13b**), as well as replacement of the entire phenothiazine moiety with an indole nucleus as in **12e** resulted in compounds with an antimycobacterial activity comparable to or better than that of **1** and **3**. In particular, the chloro-phenothiazine derivative **12b** showed a good activity against *M. bovis* BGC and *M. tuberculosis* $mc^2$7000 strains with MIC = 5.3 μg/mL and 4 μg/mL, respectively. Similarly, the methylated analogue **13b** retained a 8 μg/mL MIC value. Interestingly, the indole derivative **12e** proved to be highly active against *M. smegmatis* with MIC = 1.6 μg/mL.

The most promising compounds were then assayed against the pathogenic H37Rv strain, the drug-susceptible CF73 clinical isolate, and two MDR- clinical isolates (CF104 and CF81). Also in this case, **12b**, **12e**, and **13b** showed the best results. In particular, the chloro-derivatives **12b** and **13b** had an activity toward the CF73 and the MDR strains similar to that of **1** in the same range of concentrations (8-16 μg/mL). The indole derivative **12e** proved to be the best compound of the series, with an increased activity against both H37Rv and CF73 strains (2.9 and 1 μg/mL, respectively, in comparison to 10 and 8 μg/mL found for **1**). Moreover, **12e** also showed a similar profile against MDR-CF104 (10 vs 11 μg/mL) and a slightly improved activity against MDR-CF81 (4 vs 10 μg/mL). These data suggest that the presence of a secondary amine on the piperidine side chains could be beneficial for the anti-tubercular activity, as also observed in our previous work.[27]

Finally, to prove the effectiveness of the most active compounds, their cytotoxicity was evaluated on MRC-5 cells. As a result, **12e** showed a selectivity index 15 fold higher than that of TZ (Table 3).

Finally, the effect of TZ derivatives on the efflux pumps of the model surrogate organism *M. smegmatis* was tested in order to understand the mode of action of the new compounds. Efflux pump inhibition (EPI) is determined using a whole-cell-based assay which interrogates the total activity of the diverse sets of efflux pumps present in the cell. The EPI assay showed that some TZ derivatives are endowed with a good efflux pump inhibitory activity. However, there is a weak correlation between inhibition of bacterial growth and efflux pump inhibtion. The compounds **12b** and **12e** which showed the most promising antimycobacterial activity proved to be poor efflux pump inhibitors (Figure 2). These compounds could interfere with the cell-envelope permeability, as already hypothesized for **1**,[23] by means other than inhibiting efflux pumps. On the other hand, the piperazine derivatives **7a** and **7c** which did not show antimycobacterial activity, were found to inhibit efflux pumps better than the reference chlorpromazine. This indicates that these compounds have the potential to reverse multidrug resistance and could be promising candidates for inclusion in a combination therapy regimen owing to synergistic combinations.

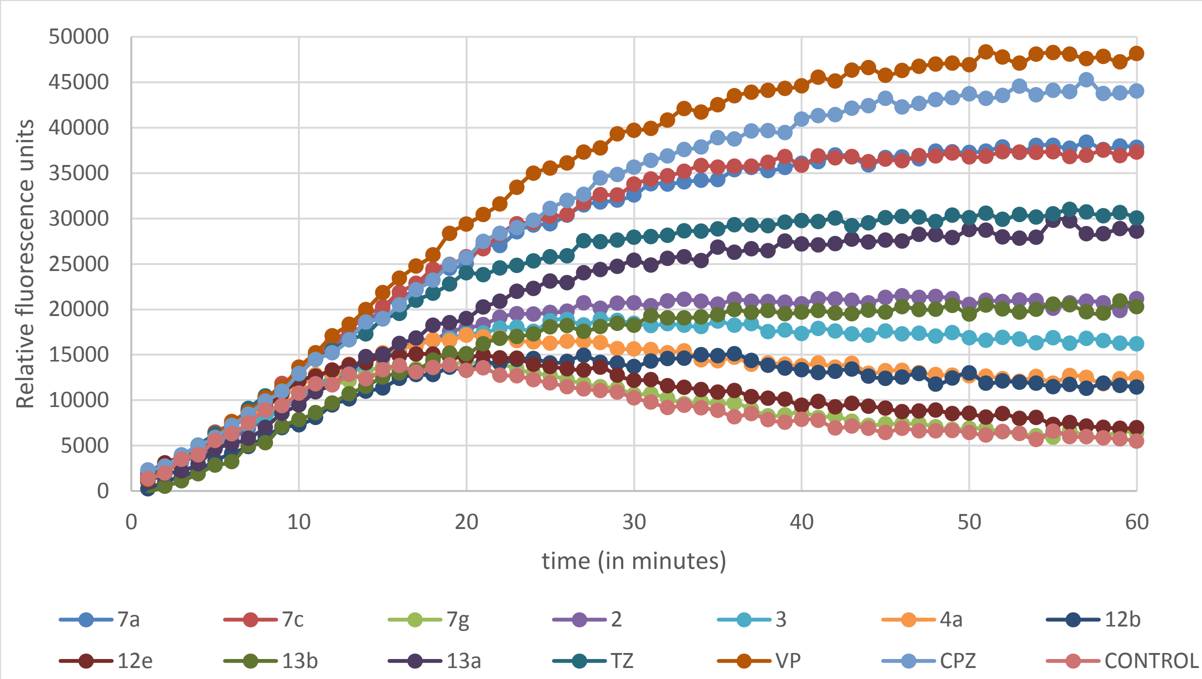


Figure 2. Efflux pump inhibition assay. Graphs showing the accumulation of ethidium bromide (EtBr) within *M. smegmatis* cells in the presence of selected compounds and positive (verapamil VP and chlorpromazine CPZ) and negative (1× PBS) controls. Low to very high inhibition of efflux (as a representation of an increased level of EtBr accumulation) are shown by relative fluorescent units. The experiments were performed in triplicate (n = 3), and the graph is plotted using the average values obtained.

3. CONCLUSIONS

A classical medicinal chemistry approach has been applied to design and synthesise a narrow library of thioridazine derivatives by structural changes made on three different molecular portions. Antimycobacterial activity of the resulting compounds showed that the piperidine-ethyl side chain is required for inhibit non-pathogenic, pathogenic and MDR mycobacterial strains. Moreover, the SMe-phenothiazine scaffold of **1** could be only replaced with the Cl-phenothiazine analogue or simplified into an indole moiety. The most active compound **12e**, bearing a demethylated piperazine ring in addition to an indole heterocycle, showed an activity profile better than that of **1** and a cytotoxicity about 15-fold lower toward MRC-5 cells.

4. MATERIAL AND METHODS

Chemistry. Materials and Methods.

^{1}H NMR and ^{13}C NMR spectra were recorded on JEOL Delta-270 or JEOL ECS-400 spectrometers operating at the frequencies indicated. Chemical shift (δ) are in ppm, referenced to tetramethylsilane. Coupling constants (J) are reported in hertz and rounded to

0.5 Hz. Splitting patterns are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br) or some combination of them. Infrared spectra were obtained using a Durascope diamond ATR system. Mass spectra (HRMS) were recorded at the EPSRC National Mass Spectrometry Service Centre on a Thermo Scientific LTQ Orbitrap XL mass spectrometer using low-resolution ESI or high-resolution nano ESI techniques. The purity of the compounds was assessed by reverse-phase liquid chromatography coupled with a mass spectrometer (Agilent series 1100 LC/MSD) with a UV detector at k = 254 nm and an electrospray ionization source (ESI). HPLC analyses were performed at 0.4 mL/min flow rate and using a binary solvent system of 95:5 methyl alcohol/water. All the solvents were of HPLC grade. Mass spectra were acquired in positive mode scanning over the mass range of 50−1500. The following ion source parameters were used: drying gas flow, 9 mL/min; nebulize pressure, 40 psig; and drying gas temperature, 350 °C. All target compounds possessed a purity of ≥95%, as verified by HPLC analyses. TLC was performed using commercially available precoated plates and visualized with UV light at 254 nm; KMnO4 was used to reveal the products. Flash column chromatography was carried out using Fluorochem Davisil 40−63 µm, 60 Å. All reactions were conducted under a nitrogen atmosphere in oven-dried glassware unless stated otherwise. THF was distilled under nitrogen from sodium using a benzophenone indicator. Dichloromethane was purchased from Aldrich. All other solvents and commercially available reagents were used as received.

Synthesis of 1-chloroethyl 2-(2-(2-(methylthio)-10H-phenothiazin-10-yl)ethyl)piperidine-1-carboxylate (2)

Thioridazine (**1**) (3.87 mmol, 1 eq.) was dissolved in a round bottomed flask containing dry DCE (20 mL) and TEA (7.74 mmol, 2 eq.). The mixture was stirred at r.t. for 20 minutes before that 1-chloroethyl chloroformate (7.74 mmol, 2 eq.) was added to the solution. The mixture was left under N_2 atmosphere at reflux for 12h. Then, the reaction mixture was quenched with 10 mL of water and extracted twice with 20 mL of EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure giving a yellow-brown crude oil. The crude product was purified by chromatography on silica gel, using hexane/EtOAc (4:1) as eluent.

Yield: 84%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.13-7.01 (m, 2H), 7.01-6.90 (m, 1H), 6.89-6.60 (m, 4H), 6.59-6.29 (m, 1H), 4.20-3.90 (m, 1H), 3.90-3.60 (m, 2H), 2.90-2.74 (m, 1H), 2.74-2.57 (m, 1H), 2.36 (s, 3H), 2.25-2.04 (m, 1H), 1.77-1.60 (m, 3H), 1.58-1.16 (m, 7H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 152.9, 145.0, 137.9, 127.8, 127.7, 127.4, 122.8, 121.0, 114.5, 83.4, 49.9, 44.5, 39.8, 29.1, 27.8, 25.4, 19.1, 16.5, 14.3 ppm. **LRMS** m/z (ES+) m/z: 463 $[M+H]^+$

Synthesis of 2-(methylthio)-10-(2-(piperidin-2-yl)ethyl)-10H-phenothiazine (3)

Derivative **2** (3.84 mmol, 1 eq.) was dissolved in MeOH (20 mL) and the solution was stirred at reflux for 12h then the reaction mixture was concentrated by reduced pressure evaporation.

Then, the reaction mixture was quenched with 20 ml of water and extracted twice with 20 mL of EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure giving a yellow-brown crude oil. The obtained product **3** was purified by chromatography on silica gel, using EtOAc/MeOH/TEA (3.9:1:0.1) as eluent.

Yield: 85% **^{1}H NMR** (400 MHz $CDCl_3$) δ 9.25 (br. s., 1H), 7.20-7.08 (m, 2H), 7.03 (d, *J* = 6.9 Hz, 1H), 6.90 (d, *J* = 7.3 Hz, 2H), 6.82 (m, 2H), 4.13-3.93 (m, 2H), 3.29 (d, *J* = 12.8 Hz, 1H), 3.02 (m, 1H), 2.68 (t, *J* = 12.1 Hz, 1H), 2.46 (s, 3H), 2.18-2.05 (m, 1H), 1.90-1.75 (m, 3H), 1.75-1.53 (m, 3H), 1.44-1.28 (m, 1H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 145.9, 144.4, 138.3, 127.8, 127.7, 127.7, 125.9, 123.2, 122.5, 121.2, 116.3, 114.5, 55.7, 44.7, 43.7, 30.7, 28.8, 22.5, 22.2, 16.4 ppm. **LRMS** m/z (ES+) m/z: 357 $[M+H]^+$

General procedure for the synthesis of thioridazine derivatives 4a-c

The 2-(methylthio)-10-(2-(piperidin-2-yl)ethyl)-10H-phenothiazine **3** (0.14 mmol, 1 eq.) was added to a round bottomed flask containing a solution of the appropriate aldehyde/ketone (0.21 mmol, 1.5 eq.) in THF (5 mL). The solution was then allowed to stir at room temperature for 30 minutes. Then, $NaBH(AcO)_3$ (0.28 mmol, 2 eq.) was added and the reaction was allowed to react for 24h at r.t.. The reaction was quenched with (20mL) NaOH 1N solution and the resulting mixture was allowed to stir for 20 minutes. Then the organic solvent was removed under reduced pressure evaporation. The residue was added with EtOAc and extracted (3 x 10 mL) and finally dried over anhydrous $MgSO_4$. The crude products **4a-c** were purified by chromatography on silica gel, using EtOAc/MeOH/TEA (3.9:1:0.1) as eluent.

10-(2-(1-Benzylpiperidin-2-yl)ethyl)-2-(methylthio)-10H-phenothiazine (4a): Yield: 67%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.28-7.17 (m, 5H) 7.14-7.10 (m, 2H), 7.03 (d, *J* = 4.0 Hz, 1H), 6.92-6.84 (m, 2H), 6.81 (d, *J* = 4.0 Hz, 2H), 3.97-3.83 (m, 3H), 3.32 (d, *J* = 8.0 Hz, 1H), 2.75-2.70 (m, 1H), 2.60-2.55 (m, 1H), 2.43 (s, 3H), 2.14 (s, 2H), 2.00-1.94 (m, 1H), 1.77-1.41 (m, 6H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 146.0, 145.1, 139.7, 137.6, 129.1, 128.8, 128.6, 128.3, 127.6, 127.6, 127.3, 126.8 125.4, 122.6, 122.3, 120.9, 115.8, 114.7, 58.4, 57.7, 55.7, 50.6, 44.2, 29.6, 24.4, 23.1, 16.6 ppm. **LRMS** m/z (ES+) m/z: 447 $[M+H]^+$. **HRMS** (ESI) m/z calcd. For $C_{27}H_{31}N_2S_2$ [M +H] 447.1923, found 447.1913.

2-(Methylthio)-10-(2-(1-propylpiperidin-2-yl)ethyl)-10H-phenothiazine (4b): Yield: 62%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.15-7.11 (m, 2H), 7.03 (d, *J* = 4.0 Hz, 1H), 6.92-6.88 (m, 2H), 6.80 (d, *J* = 4.0 Hz, 2H), 3.94-3.99 (m, 1H), 3.83-3.79 (m, 1H), 2.82-2.77 (m, 1H), 2.51-2.48 (m, 2H), 2.45 (s, 3H), 2.33-2.22 (m, 2H), 2.16-2.06 (m, 1H), 1.85-1.56 (m, 4H), 1.43-1.29 (m, 5H), 0.75 (t, *J* = 8.0 Hz, 3H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 145.8, 145.0, 137.6, 127.6, 127.5, 127.3, 125.4, 122.6, 122.3, 120.9, 115.8, 114.7, 57.9, 55.7, 51.4, 44.4, 30.2, 27.9, 25.2, 23.3, 18.9, 16.6, 12.0 ppm. **LRMS** m/z (ES+) m/z: 399 $[M+H]^+$. **HRMS** (ESI) m/z calcd. for $C_{23}H_{31}N_2S_2$ [M +H] 399.1923, found 399.1916.

Synthesis of 10-(2-(1-isopropylpiperidin-2-yl)ethyl)-2-(methylthio)-10H-phenothiazine (4c): Yield: 68%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.15-7.11 (m, 2H), 7.03 (d, *J* = 4.0 Hz, 1H), 6.92-6.88 (m, 2H), 6.80 (d, *J* = 4.0 Hz, 2H), 3.96-3.98 (m, 1H), 3.83-3.79 (m, 1H), 3.15 (t, *J* = 8.0 Hz, 1H), 2.81-2.75 (m, 1H), 2.62-2.59 (m, 1H), 2.44 (s, 3H), 2.20-2.03 (m, 2H), 1.85-1.24 (m, 7H), 1.05 (d, *J* = 8.0 Hz, 3H), 0.77 (d, *J* = 8.0 Hz, 3H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 145.8, 127.6, 127.5, 127.3, 122.6, 120.9, 115.7, 114.7, 56.1, 44.1, 43.9, 31.0, 28.5, 26.0, 24.0, 21.8, 16.6, 13.9 ppm. **LRMS** m/z (ES+) m/z: 399 $[M+H]^+$. **HRMS** (ESI) m/z calcd. for $C_{23}H_{31}N_2S_2$ [M +H] 399.1923, found 399.1916.

General procedure for the synthesis of compound (6a-c)

The appropriate 2-substituted phenothiazine **5a-c** (0.42 mmol, 1eq.) was added to 5 mL of DMF in a double neck round bottomed flask. NaH (0.46 mmol, 1.1 eq.) was added to the stirring solution at 0°C, and the mixture was allowed to reach r.t stirring for 20 minutes. Then, 1-bromo-3-chloropropane (0.46 mmol 1.1eq) was added to the stirring solution. The reaction mixture was allowed to stir under N_2 atmosphere for 12h at r.t. before being quenched with 10 mL of water and extracted twice with 20 mL of EtOAc. The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure giving a yellow-brown crude oil. The obtained product was purified by chromatography on silica gel, using hexane/EtOAc (4:1) as eluent.

10-(3-Chloropropyl)-2-(methylthio)-10H-phenothiazine (6a): Yield: 93%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.18-7.14 (m, 2H), 7.06 (d, *J* = 8.0 Hz, 1H), 6.95-6.88 (m, 2H), 6.84-6.81 (m, 2H), 4.06 (t, *J* = 8.0 Hz, 2H), 3.65 (t, *J* = 8.0 Hz, 2H), 2.46 (s, 3H), 2.22 (t, *J* = 8.0 Hz, 2H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 145.6, 145.9, 137.8, 127.8, 127.7, 127.5, 125.8, 123.0, 122.7, 121.1, 115.9, 114.6, 44.1, 42.5, 29.7, 16.5 ppm. **LRMS** m/z (ES+) m/z: 322 $[M+H]^+$.

2-Chloro-10-(3-chloropropyl)-10H-phenothiazine (6b): Yield: 84%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.19-7.14 (m, 2H) 7.04 (d, *J* = 8.0 Hz, 1H), 6.95 (t, *J* = 8.0 Hz, 1H), 6.91-6.88 (m, 2H), 6.86 (s, 1H), 4.04 (t, *J* = 8.0 Hz, 2H), 3.65 (t, *J* = 8.0 Hz, 2H), 2.25-2.18 (m, 2H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 146.5, 144.4, 133.4, 128.2, 127.8, 127.6, 125.5, 124.2, 123.3, 122.7, 116.0, 115.9, 44.1, 42.3, 29.5 ppm. **LRMS** m/z (ES+) m/z: 332 $[M+Na]^+$

10-(3-Chloropropyl)-10H-phenothiazine (6c): Yield: 82%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.19-7.15 (m, 4H), 6.96-6.89 (m, 4H), 4.07 (t, *J* = 8.0 Hz, 2H), 3.66 (t, *J* = 8.0 Hz, 2H), 2.23 (t, *J* = 8.0 Hz, 2H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 145.2, 127.8, 127.4, 125.8, 122.9, 115.7, 44.0, 42.6, 29.7 ppm. **LRMS** m/z (ES+) m/z: 276 $[M+H]^+$

General procedure for the synthesis of thioridazine derivatives (7a-i)

The appropriate chloro derivative **6a-c** (0.18 mmol, 1 eq.) was dissolved in a round bottomed flask containing dry DMF (10 mL) and DIPEA (0.19 mmol, 1.1 eq.). The appropriate amine (*N*-substituted piperazine or thiomorpholine) (0.72 mmol, 4 eq.) was then added to the solution followed by NaI (5 mg, 0.036 mmol, 0.2 eq.). The mixture was left under N_2 atmosphere at 150 °C for 3 h, after which time it was added with water (10 mL) and extracted twice with EtOAc (20 mL). The combined organic layers were washed with brine, dried over

Na_2SO_4 and concentrated under reduced pressure. The obtained product was purified by chromatography on silica gel, using EtOAc/MeOH/TEA (3.9:1:0.1) as eluent.

10-(3-(4-Methylpiperazin-1-yl)propyl)-10H-phenothiazine (7a):[28] **Yield:** 99%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.12 (t, *J* = 8.0 Hz, 4H), 6.90 (t, *J* = 8.0 Hz, 4H), 3.90 (t, *J* = 8.0 Hz, 2H), 2.46 (t, *J* = 8.0 Hz, 2H), 2.42-2.34 (m, 8H), 2.26 (s, 3H), 1.94 (d, *J* = 8.0 Hz, 2H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 145.2, 127.5, 127.3, 125.1, 122.5, 115.6, 55.7, 55.1, 53.2, 46.0, 45.4, 24.5 ppm. **LRMS** m/z (ES+) m/z: 340 $[M+H]^+$.

10-(3-(4-Phenylpiperazin-1-yl)propyl)-10H-phenothiazine (7b): Yield: 62%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.25 (t, *J* = 8.0 Hz, 2H), 7.13 (d, *J* = 8.0 Hz, 4H), 6.92-6.88 (m, 6H), 6.84 (t, *J* = 8.0 Hz, 1H), 3.95 (t, *J* = 8.0 Hz, 2H), 3.14 (t, *J* = 8.0 Hz, 4H), 2.57 (t, *J* = 8.0 Hz, 4H), 2.53 (t, *J* = 8.0 Hz, 2H), 2.02-1.95 (m, 2H) ppm. **^{13}C** NMR (100 MHz $CDCl_3$) δ 151.4, 145.3, 129.2, 127.5, 127.3, 125.2, 122.5, 119.7, 116.1, 115.6, 55.7, 53.4, 49.2, 45.3, 24.4 ppm. **LRMS** m/z (ES+) m/z: 402 $[M+H]^+$. **HRMS** (ESI) m/z calcd. for $C_{25}H_{28}N_3S$ [M +H] 402.1998, found 402.1995.

10-(3-(4-Methylpiperazin-1-yl)propyl)-2-(methylthio)-10H-phenothiazine (7c): Yield: 99%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.11-7.09 (m, 2H), 7.01 (d, *J* = 8.0 Hz, 1H), 6.90-6.85 (m, 2H), 6.80-6.77 (m, 2H), 3.89 (t, *J* = 8.0 Hz, 2H), 2.50-2.33 (m, 8H), 2.46 (t, *J* = 8.0 Hz, 2H), 2.44 (s, 3H), 2.26 (s, 3H), 1.94-1.92 (m, 2H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 145.2, 144.9, 137.5, 127.6, 127.5, 127.3, 125.2, 122.6, 122.2, 120.8, 115.8, 114.7, 55.6, 55.1, 53.2, 46.0, 45.3, 24.4, 16.6 ppm. **LRMS** m/z (ES+) m/z: 386 $[M+H]^+$. **HRMS** (ESI) m/z calcd. for $C_{21}H_{28}N_3S_2$ [M +H] 386.1719, found 386.1739.

2-(Methylthio)-10-(3-(4-phenylpiperazin-1-yl)propyl)-10H-phenothiazine (7d): Yield: 77%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.24 (t, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 7.03 (d, *J* = 8.0 Hz, 1H), 6.92-6.88 (m, 4H), 6.85-6.80 (m, 3H), 3.94 (t, *J* = 8.0 Hz, 2H), 3.14 (t, *J* = 8.0 Hz, 4H), 2.56 (t, *J* = 8.0 Hz, 4H), 2.51 (t, *J* = 8.0 Hz, 2H), 2.45 (s, 3H), 1.99-1.96 (m, 2H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 151.4, 145.7, 145.02, 137.6, 129.2, 127.6, 127.5, 127.3, 125.3, 122.7, 122.3, 120.9, 119.7, 116.1, 115.8, 114.8, 55.7, 53.4, 49.2, 45.3, 24.4, 16.6 ppm. **LRMS** m/z (ES+) m/z: 448 $[M+H]^+$. **HRMS** (ESI) m/z calcd. for $C_{26}H_{30}N_3S_2$ [M +H] 448.1876, found 448.1866.

2-(Methylthio)-10-(3-thiomorpholinopropyl)-10H-phenothiazine (7e): Yield: 99%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.14-7.03 (m, 2H), 7.01 (d, *J* = 8.0 Hz, 1H), 6.91-6.86 (m, 2H), 6.80-6.77 (m, 2H), 3.90 (t, *J* = 8.0 Hz, 2H), 2.63-2.57 (m, 8H), 2.45 (s, 3H), 1.89 (t, *J* = 8.0 Hz, 2H) 1.34-1.22 (m, 2H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 145.7, 144.9, 137.6, 127.6, 127.5, 127.3, 122.6, 122.2, 120.8, 115.9, 114.8, 56.1, 55.2, 45.1, 28.1, 24.1, 16.6 ppm. **LRMS** m/z (ES+) m/z: 389 $[M+H]^+$. **HRMS** (ESI) m/z calcd. for $C_{20}H_{25}N_2S_3$ [M +H] 389.1174, found 389.1172.

10-(3-(4-Adamantan-1-yl)piperazin-1-yl)propyl)-2-(methylthio)-10H-phenothiazine (7f): Yield: 71%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.17-7.07 (m, 2H), 7.02 (d, *J* = 7.8 Hz, 1H), 6.92-6.85 (m, 2H), 6.84-6.76 (m, 2H), 3.89 (t, *J* = 6.9 Hz, 2H), 2.56-2.26 (m, 12H), 2.07-1.88 (m, 8H), 1.88-1.74 (m, 4H), 1.71-1.53 (m, 4H), 1.35 (d, *J* = 11.9 Hz, 2H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 145.8, 144.9, 137.6, 127.6, 127.5, 127.3, 125.3, 122.7, 122.3, 120.8, 115.9, 114.7, 55.4, 54.3, 53.2, 46.7, 45.4, 45.2, 44.0, 43.9, 41.0, 38.7, 38.0, 36.8, 36.7, 29.7, 24.3, 16.6 ppm. **LRMS** m/z (ES+) m/z: 506 $[M+H]^+$. **HRMS** (ESI) m/z calcd. for $C_{30}H_{40}N_3S_2$ [M +H] 506.2658, found 506.2644.

10-(3-(4-(Adamantan-2-yl)piperazin-1-yl)propyl)-2-(methylthio)-10H-phenothiazine (7g): Yield: 72%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.15-7.04 (m, 2H), 7.01 (d, *J* = 7.8 Hz, 1H), 6.92-6.81 (m, 2H), 6.81 - 6.73 (m, 2H), 3.87 (t, *J* = 6.6 Hz, 2H), 3.58-3.41 (m, 1H), 3.38-3.25 (m, 1H), 2.70 (s, 3H), 2.64-2.59 (m, 2H), 2.59-2.55 (m, 2H), 2.55-2.49 (m, 2H), 2.49-2.44 (m, 3H), 2.43 (s, 3H), 2.07 (m, 5H), 1.97-1.87 (m, 2H), 1.73 (m, 5H), 1.64 (d, *J* = 2.3 Hz, 3H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 145.8, 144.9, 137.6, 127.6, 127.5, 127.3, 125.2, 122.6, 122.2, 120.8, 115.8, 114.7, 67.8, 56.0, 54.0, 49.6, 45.6, 40.6, 37.9, 37.3, 31.4, 29.1, 28.9, 27.6, 27.4, 24.4, 16.6 ppm. **LRMS** m/z (ES+) m/z: 506 $[M+H]^+$. **HRMS** (ESI) m/z calcd. for $C_{30}H_{40}N_3S_2$ [M +H] 506.2658, found 506.2648.

2-Chloro-10-(3-(4-phenylpiperazin-1-yl)propyl)-10H-phenothiazine (7h): Yield: 57%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.24 (t, *J* = 8.0 Hz, 2H), 7.17-7.11 (m, 2H), 7.01 (d, *J* = 8.0 Hz, 1H), 6.94-6.63 (m, 7H), 3.92 (t, *J* = 8.0 Hz, 2H), 3.15 (t, *J* = 8.0 Hz, 4H), 2.57 (t, *J* = 8.0 Hz, 4H), 2.51 (t, *J* = 8.0 Hz, 2H), 2.00-1.96 (m, 2H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 151.4, 146.6, 144.6, 133.3, 129.2, 128.0, 127.6, 127.5, 124.8, 123.6, 123.0, 122.3, 119.8, 116.1, 115.9, 55.6, 53.5, 49.2, 45.4, 24.3 ppm. **LRMS** m/z (ES+) m/z: 436 $[M+H]^+$. **HRMS** (ESI) m/z calcd. For [M +H], found.

2-Chloro-10-(3-thiomorpholinopropyl)-10H-phenothiazine (7i): Yield: 90% **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.15-7.08 (m, 2H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.93-6.81 (m, 4H), 3.88 (t, *J* = 8.0 Hz, 2H), 2.66-2.57 (m, 8H), 2.45 (t, *J* =8.0 Hz, 2H), 1.91-1.85 (m, 2H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 146.5, 144.6, 133.2, 127.9, 127.6, 127.5, 124.8, 123.5, 122.9, 122.3, 115.9, 56.0, 55.8, 45.1, 28.1, 24.0 ppm. **LRMS** m/z (ES+) m/z: 377 $[M+H]^+$. **HRMS** (ESI) m/z calcd. For [M +H], found.

2-Chloro-1-(2-(methylthio)-10H-phenothiazin-10-yl)ethanone (8)

The phenothiazine **5a** (1.22 mmol, 1 eq.) was dissolved to 15 mL of DMF in a double neck round bottomed flask. NaH (1.83 mmol, 1.5 eq.) was added to the stirring solution at 0°C, which then was allowed to reach r.t. under stirring for 20 minutes. Then, 2-chloroacetyl chloride (3.66 mmol 3 eq.) was added and the reaction mixture was stirred under N_2 atmosphere for 3h at r.t.. The reaction mixture was then quenched with water (10 mL) and extracted twice with EtOAc (20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure giving a yellow-brown crude oil. The obtained product was purified by chromatography on silica gel, using hexane/EtOAc (3:2) as eluent.

Yield: 58%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.53 (d, *J* = 8.0 Hz, 1H), 7.47-7.43 (m, 2H), 7.36-7.31 (m, 2H), 7.27-7.23 (m, 1H), 7.14-7.11 (m, 1H), 4.16 (d, *J* = 8.0 Hz, 2H), 2.49 (s, 3H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 166.3, 138.8, 138.3, 137.5, 128.3, 128.2, 127.7, 127.5, 126.5, 125.7, 124.1, 40.7, 15.9 ppm. **LRMS** m/z (ES+) m/z: 322 $[M+H]^+$.

Synthesis of 2-(4-methylpiperazin-1-yl)-1-(2-(methylthio)-10H-phenothiazin-10-yl)ethanone (9a)

The 1-methylpiperazine (0.63 mmol, 4 eq.) was dissolved in a round bottomed flask containing dry DMF (5 mL) and DIPEA (0.17 mmol, 1.1 eq.) The mixture was stirred at r.t for 20 minutes, then, 2-chloro-1-(2-(methylthio)-10H-phenothiazin-10-yl)ethanone (**8**) (0.15 mmol, 1 eq.) was added to the solution followed by NaI (0.03 mmol, 0.2 eq.). The mixture was left under N_2 atmosphere at 153 °C for 3 h. Then, the reaction mixture was quenched with water (10 mL) and extracted twice with EtOAc (20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure giving a yellow-brown crude oil. The obtained product was purified by chromatography on silica gel, using EtOAc/MeOH/TEA (3.9:1:0.1) as eluent.

Yield: 96%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.51-7.47 (m, 2H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.32-7.26 (m, 2H), 7.22-7.18 (m, 1H), 7.10-7.08 (m, 1H), 3.30-3.31 (m, 2H), 2.47 (s, 3H), 2.44-2.27 (8H), 2.22 (s, 3H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 168.6, 139.3, 138.6, 137.9, 128.0, 127.9, 127.0, 126.9, 125.1, 124.9, 60.4, 54.9, 52.9, 45.9, 16.2 ppm. **LRMS** m/z (ES+) m/z: 386 $[M+H]^+$. **HRMS** (ESI) m/z calcd. for $C_{20}H_{24}N_3S_2O$ [M +H], 386.1355 found 386.1351.

Synthesis of 1-(2-(methylthio)-10H-phenothiazin-10-yl)-2-(piperidin-1-yl)ethanone (9b)

Piperidine (1.22 mmol, 4 eq.) was added to a round bottomed flask containing DMF (5 mL) and DIPEA (0.31 mmol, 1 eq.) and the mixture was allowed to stir at room temperature for 30 minutes before 2-chloro-1-(2-(methylthio)-10H-phenothiazin-10-yl)ethanone (**8**) (0.31 mmol, 1 eq.) was added to the solution. The reaction was allowed to stir for 3h at 150 °C. The reaction mixture was then quenched with water (10 mL) and extracted twice with EtOAc (20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure giving a yellow-brown crude oil. The obtained product was purified by chromatography on silica gel, using EtOAc/MeOH/TEA (3.9:1:0.1) as eluent.

Yield: 91% **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.51-7.47 (m, 2H), 7.40 (d, *J* = 8.0 Hz, 1H), 7.30-7.24 (m, 1H), 7.19 (t, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 12.0 Hz, 2H), 3.24-3.23 (d, *J* = 8.0 Hz, 2H), 2.47 (s, 3H), 2.35 (m, 4H), 1.46-1.41 (m, 4H), 1.32-1.29 (m, 2H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 163.7, 138.9, 135.8, 135.7, 132.3, 132.2, 126.7, 126.7, 122.2, 118.0, 116.2, 56.8, 56.1, 25.2, 24.2, 16.0 ppm. **LRMS** m/z (ES+) m/z: 371 $[M+H]^+$ **HRMS** (ESI) m/z calcd. For [M +H], found.

Synthesis of *t*-butyl 2-(2-bromoethyl)piperidine-1-carboxylate (11)

The 2-(piperidin-2-yl)ethanol **10** (1.55 mmol 1 eq.) was added to 10 mL mixture (1:1) of CH_2Cl_2 and Na_2CO_3 aqueous solution (10 mL) in a round bottomed flask. Di-*t*-butyl dicarbonate (1.70 mmol, 1.1 eq.) was added to the stirring solution. The reaction mixture was stirred for 24h at room temperature. Then, the reaction mixture was diluted with 10 mL of water and extracted once with 10 mL of CH_2Cl_2 and twice with 20 mL of EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure giving a yellow crude oil. The crude product was purified by chromatography on silica gel, using hexane/EtOAc (3:2) as eluent affording the N-Boc-protected amino alcohol. **Yield:** 98% **^{1}H NMR** (400 MHz $CDCl_3$) δ 4.35-4.19 (m, 1H) 3.85-3.80 (m, 2H), 3.48-3.42 (m, 1H), 3.27 (br s, 1H), 2.59-2.52 (m, 1H), 1.81 (t, *J* = 12.0 Hz, 1H), 1.64-1.54 (m, 1H), 1.51-1.37 (m, 5H), 1.37 (s, 9H), 1.29-1.25 (m, 1H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 155.0, 80.2, 58.7, 46.1, 39.4, 32.5, 28.5, 28.4, 25.6, 19.0 ppm. **LRMS** m/z (ES+) m/z: 230 $[M+Na]^+$

To a solution of the above synthesised N-Boc-amino alcohol (0.43 mmol 1eq.) in CH_2Cl_2 (10 mL) was added PPh_3 (0.47 mmol, 1.1 eq.) followed by a solution of CBr_4 (0.47mmol, 1.1 eq.) in 20 mL of CH_2Cl_2 at r.t. and the mixture was allowed to stir for 45 min. Then, the reaction mixture was concentrated under reduced pressure giving a yellow crude oil. The obtained product was then immediately purified by chromatography on silica gel, using hexane/EtOAc (9:1) as eluent. The pure product **11** was obtained as a yellow oil. **Yield:** 79% **^{1}H NMR** (400 MHz $CDCl_3$) δ 4.35-4.31 (m, 1H) 4.02-3.84 (m, 1H), 3.33-3.19 (m, 2H), 2.70-2.64 (t, *J* = 12.0 Hz, 1H), 2.33-2.23 (m, 1H), 1.90-1.64 (m, 1H), 1.63-1.43 (m, 5H), 1.40 (s, 9H), 1.38-1.31 (m, 1H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 155.2, 79.6, 49.5, 38.7, 33.6, 30.3, 28.7, 28.5, 25.5, 19.2 ppm. **LRMS** m/z (ES+) m/z: 293 $[M+H]^+$

General procedure for the synthesis of Boc-protected thioridazine derivatives (S12a-e)

The appropriate 2-substituted phenothiazine (**5a-c**) or carbazole, indole, or benzimidazole (0.42 mmol, 1eq.) was added to 10 mL of DMF in a double neck round bottomed flask. NaH (0.52 mmol, 1.2 eq.) was added to the stirring solution at 0°C, which then was allowed to reach r.t stirring for 20 minutes. Then, *t*-butyl 2-(2-bromoethyl)piperidine-1-carboxylate (**11**) (0.52 mmol 1.2eq.) and NaI (0.02 mmol, 0.1eq) were added to the stirring solution. The reaction mixture was allowed to stir under N_2 atmosphere for 12h at r.t. Then, the reaction mixture was quenched with 20 mL of water and extracted twice with 20 mL of EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure giving a yellow-brown crude oil. The crude product was purified by chromatography on silica gel, using hexane/EtOAc (4:1) as eluent.

***t*-Butyl 2-(2-(10H-phenothiazin-10-yl)ethyl)piperidine-1-carboxylate (S12a): Yield:** 45%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.16-7.12 (m, 4H), 6.93-6.89 (m, 2H), 6.84 (d, *J* = 8.0 Hz, 2H), 4.45-4.35 (m, 1H), 4.08-3.96 (m, 1H), 3.92-3.74 (m, 2H), 3.38-3.28 (m, 1H), 2.83-2.69 (m, 1H), 2.37-2.18 (m, 1H), 1.91-1.85 (m, 1H), 1.67-1.49 (m, 5H), 1.40 (s, 9H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 155.1, 145.3, 127.6, 127.3, 125.1, 122.5, 115.3, 79.4, 49.5, 38.7, 33.6, 30.3, 28.7, 28.5, 25.5, 19.2 ppm. **LRMS** m/z (ES+) m/z: 411 $[M+H]^+$

***t*-Butyl 2-(2-(2-chloro-10H-phenothiazin-10-yl)ethyl)piperidine-1-carboxylate (S12b): Yield:** 61%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.17-7.11 (m, 2H) 7.02 (d, *J* = 8.0 Hz, 1H), 6.95-6.83 (m, 3H), 6.78 (d, *J* = 4.0 Hz, 1H), 4.44-4.39 (m, 1H), 4.38-4.35 (m, 1H), 4.05-4.00 (m, 1H), 3.88-3.79 (m, 2H), 2.78 (t, *J* = 12 Hz, 1H), 2.22-2.16 (m, 1H), 1.90-1.81 (m, 1H), 1.68-

1.45 (m, 5H), 1.42 (s, 9H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 155.2, 146.7, 144.6, 133.4, 128.1, 127.5, 124.9, 123.0, 122.4, 115.7, 79.6, 48.8, 44.8, 39.1, 29.3, 28.5, 27.7, 25.6, 19.3 ppm. **LRMS** m/z (ES+) m/z: 468 $[M+Na]^+$

***t*-Butyl 2-(2-(9H-carbazol-9-yl)ethyl)piperidine-1-carboxylate (S12c): Yield:** 61% **^{1}H NMR** (400 MHz CD_3OD) δ 8.37 (d, *J* = 7.8 Hz, 2H), 7.87-7.64 (m, 4H), 7.60-7.35 (m, 2H), 4.74 (m, 1H), 4.69-4.59 (m, 1H), 4.36-4.18 (m, 1H), 3.69-3.60 (m, 2H), 3.10-3.07 (m, 1H), 2.73-2.53 (m, 1H), 2.22 (m, 1H), 2.00-1.86 (m, 5H), 1.85-1.76 (m, 9H) ppm. **^{13}C NMR** (100 MHz CD_3OD) δ 155.8, 140.6, 140.5, 125.9, 125.6, 123.4, 123.3, 120.4, 120.0, 119.1, 118.7, 110.8, 108.7, 80.1, 78.6, 40.2, 33.2, 28.5, 27.9, 27.8, 25.7, 19.1, 19.0 ppm. **LRMS** m/z (ES+) m/z: 401 $[M+Na]^+$

***t*-Butyl 2-(2-(1H-benzo[d]imidazol-1-yl)ethyl)piperidine-1-carboxylate (S12d): Yield:** 63% **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.91 (s, 1H), 7.84-7.67 (m, 1H), 7.39-7.31 (m, 1H), 7.31-7.09 (m, 2H), 4.49-4.19 (m, 1H), 4.10-3.90 (m, 2H), 2.81-2.65 (m, 1H), 2.38-2.18 (m, 1H), 1.96-1.77 (m, 1H), 1.72-1.44 (m, 7H), 1.44-1.29 (m, 9H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 155.2, 144.0, 143.2, 133.7, 122.9, 122.1, 120.6, 109.5, 79.9, 60.4, 42.4, 30.1, 28.9, 28.5, 25.5, 19.2, 14.2 ppm. **LRMS** m/z (ES+) m/z: 330 $[M+H]^+$

***t*-Butyl 2-(2-(1H-indol-1-yl)ethyl)piperidine-1-carboxylate (S12e): Yield:** 42%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.62 (d, *J* = 8.0 Hz, 1H) 7.31 (d, *J* = 8.0 Hz, 1H), 7.22-7.18 (m, 1H), 7.12-7.07 (m, 2H), 6.48 (d, *J* = 4.0 Hz, 1H), 4.38-4.33 (m, 1H), 4.15-4.03 (m, 3H), 2.81 (t, *J* = 8.0 Hz, 1H), 2.31-2.21 (m, 1H), 1.93-1.86 (m, 1H), 1.68-1.58 (m, 6H), 1.45 (s, 9H) ppm **^{13}C NMR** (100 MHz $CDCl_3$) δ 155.2, 135.8, 128.8, 127.9, 121.5, 121.1, 119.4, 109.2, 101.2, 79.7, 43.8, 30.7, 29.0, 28.6, 25.6, 19.2 ppm. **LRMS** m/z (ES+) m/z: 352 $[M+Na]^+$

General procedure for the synthesis of thioridazine derivatives (12a-e)

The appropriate Boc-protected compound (**S12a-e**) (0.24 mmol, 1 eq.) was added to a round bottom flask containing 5 ml HCl saturated solution in EtOAc. The reaction mixture was allowed to stir at room temperature for 24h. The solvent was removed under reduced pressure giving a white solid as product of the reaction. The solid products **12a-e** were washed several times with cold Et_2O.

10-(2-(piperidin-2-yl)ethyl)-10H-phenothiazine (12a): Yield: 99% **^{1}H NMR** (400 MHz $CDCl_3$) δ 9.41 (br. s., 1H), 9.24 (br. s., 1H), 7.15-7.12 (m, 4H), 6.92-6.86 (m, 4H), 3.97-3.92 (m, 2H), 3.49-3.45 (m, 1H), 3.08-3.05 (m, 1H), 2.73-2.57 (m, 1H), 2.45-2.43 (m, 1H), 2.18-2.06 (m, 1H), 1.93-1.74 (m, 3H), 1.69-1.60 (m, 2H), 1.44-1.34 (m, 1H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 145.3, 127.7, 127.4, 125.4, 122.7, 115.6, 55.6, 46.8, 44.3, 33.7, 32.4, 25.9, 24.5 ppm. **LRMS** m/z (ES+) m/z: 312 $[M+H]^+$

Synthesis of 2-chloro-10-(2-(piperidin-2-yl)ethyl)-10H-phenothiazine (12b): Yield: 94% **^{1}H NMR** (400 MHz $CDCl_3$) δ 9.54 (br s, 1H), 9.34 (br s, 1H), 7.19 (t, *J* = 8.0Hz 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 7.04 (d, *J* = 8.0 Hz, 1H), 6.95-6.89 (m, 4H), 4.07-3.96 (m, 2H), 3.31 (d, *J* = 8.0 Hz, 1H), 3.12-3.04 (m, 1H), 2.77-2.68 (m, 1H), 2.50-2.42 (m, 1H), 2.13-2.09 (m, 1H), 1.85-1.69 (m, 6H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 146.6, 144.5, 133.3, 128.0, 127.6, 127.5, 125.1, 123.9, 123.1, 122.5, 115.9, 55.4, 46.7, 44.4, 33.5, 29.7, 25.8, 24.4 ppm. **LRMS** m/z (ES+) m/z: 367 $[M+Na]^+$

Synthesis of 9-(2-(piperidin-2-yl)ethyl)-9H-carbazole (12c): Yield: 95% **^{1}H NMR** (400 MHz $CDCl_3$) δ 9.66 (br s, 1H), 9.41 (br s, 1H), 8.02 (d, *J* = 8.0, 2H), 7.46-7.37 (m, 4H), 7.17 (t, *J* = 8.0, 2H), 4.51-4.43 (m, 1H), 4.31-4.25 (m, 1H), 3.38-3.36 (m, 1H), 3.02-2.90 (m, 1H), 2.70-2.67 (m, 1H), 2.49-2.48 (m, 1H), 2.18-2.12 (m, 1H), 1.88-1.61 (m, 6H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 140.0, 126.1, 122.9, 120.4, 119.3, 108.8, 55.5, 44.9, 39.5, 32.7, 30.0, 22.3, 22.0 ppm. **LRMS** m/z (ES+) m/z: 279 $[M+H]^+$

Synthesis of 1-(2-(piperidin-2-yl)ethyl)-1H-benzo[d]imidazole (12d): Yield: 90% **^{1}H NMR** (400 MHz $CDCl_3$) δ 10.17 (s 1H), 9.68 (br s, 2H), 7.82 (t, *J* = 8.0 Hz, 2H), 7.44-7.36 (m, 2H), 5.01-4.79 (m, 2H), 3.46-3.43 (m, 1H), 3.36-3.25 (m, 1H), 2.98-2.94 (m, 1H), 2.85-2.71 (m, 1H), 2.50-2.36 (m, 1H) 1.90-1.75 (m, 6H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 140.9, 130.7, 126.9, 126.7, 115.6, 112.7, 54.1, 45.0, 43.7, 33.4, 28.7, 22.3, 20.9 ppm. **LRMS** m/z (ES+) m/z: 230 $[M+H]^+$

Synthesis of 1-(2-(piperidin-2-yl)ethyl)-1H-indole (12e): Yield: 94% **^{1}H NMR** (400 MHz CD_3OD) δ 7.42-7.34 (m, 1H), 7.24-7.19 (m, 1H), 7.10-7.01 (m, 2H), 6.90-6.80 (m, 2H), 4.12-4.01 (m, 2H), 3.39-3.24 (m, 1H), 2.95-2.89 (m, 2H), 2.82-2.73 (m, 1H), 2.75-2.59 (m, 1H), 2.49-2.40 (m, 1H), 2.38-2.29 (m, 2H), 1.84-1.67 (m, 3H) ppm. **^{13}C NMR** (100 MHz $(CD_3)_2SO$) δ 135.8, 127.9, 121.8, 121.1, 119.6, 119.1, 109.7, 54.2, 45.2, 43.6, 33.3, 28.8, 22.1, 20.8 ppm. **LRMS** m/z (ES+) m/z: 229 $[M+H]^+$

General procedure for the synthesis of thioridazine derivatives (13a-d)

Compounds **(12a-d)** (0.14 mmol, 1 eq.) were added to a round bottom flask containing THF (5 mL) and formaldehyde aqueous solution 37% w/v (0.28 mmol, 2 eq.). The solution was then allowed to stir at room temperature for 30 minutes. Then, $NaBH(AcO)_3$ (0.28 mmol, 2.0 eq.) was added. The reaction was then stirred for 24h at r.t. after which time the solution was quenched with (20mL) NaOH 1N aqueous solution. The resulting mixture was stirred for further 20 minutes and then the organic solvent was removed through reduced pressure evaporation. The residue was diluted with EtOAc, extracted twice with EtOAc (10 mL) and dried over anhydrous $MgSO_4$. The solvent was concentrated under reduced pressure and the obtained product was purified by chromatography on silica gel, using EtOAc/MeOH/TEA (3.9:1:0.1) as eluent.

10-(2-(1-Methylpiperidin-2-yl)ethyl)-10H-phenothiazine (13a): Yield: 47%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.19-7.07 (m, 4H), 6.95-6.85 (m, 4H), 3.95 (ddd, *J* = 13.9, 8.6, 5.5 Hz, 1H), 3.89-3.77 (m, 1H), 2.89-2.72 (m, 1H), 2.22-2.16 (m, 3H), 2.16-1.99 (m, 3H), 1.93-1.80 (m, 1H), 1.71 (d, *J* = 10.1 Hz, 1H), 1.64-1.50 (m, 2H), 1.34-1.18 (m, 3H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 145.4, 127.6, 127.3, 125.4, 122.5, 115.6, 62.2, 57.0, 43.9, 43.1, 30.8, 30.0, 25.7, 24.2 ppm. **LRMS** m/z (ES+) m/z: 325 $[M+H]^+$. **HRMS** (ESI) m/z calcd. for $C_{20}H_{25}N_2S$ [M +H] 325.1733, found 325.1735.

2-Chloro-10-(2-(1-methylpiperidin-2-yl)ethyl)-10H-phenothiazine (13b): Yield: 38%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.11-7.02 (m, 2H), 6.94 (d, *J* = 8.3 Hz, 1H), 6.85 (t, *J* = 7.4 Hz, 1H), 6.82-6.73 (m, 3H), 3.91-3.78 (m, 1H), 3.78-3.63 (m, 1H), 2.85-2.68 (m, 1H), 2.14 (s, 3H), 2.07-1.95 (m, 3H), 1.85-1.72 (m, 1H), 1.69-1.58 (m, 2H), 1.56-1.46 (m, 2H), 1.28-1.11 (m, 2H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 147.5, 145.2, 133.8, 128.4, 128.1, 127.8, 126.0, 125.1, 123.6, 122.8, 116.8, 116.5, 62.5, 57.1, 44.4, 42.3, 30.9, 29.7, 25.6, 24.1 ppm. **LRMS** m/z (ES+) m/z: 359 $[M+H]^+$. **HRMS** (ESI) m/z calcd. for $C_{20}H_{24}ClN_2S$ [M +H] 359.1343, found 359.1347.

9-(2-(1-Methylpiperidin-2-yl)ethyl)-9H-carbazole (13c): Yield: 67%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 8.10 (d, *J* = 7.3 Hz, 2H), 7.52-7.32 (m, 4H), 7.29-7.06 (m, 2H), 4.52-4.38 (m, 1H), 4.38-4.22 (m, 1H), 2.97-2.82 (m, 1H), 2.33 (s, 3H), 2.17-2.06 (m, 2H), 2.06-1.94 (m, 2H), 1.79 (d, *J* = 11.9 Hz, 2H), 1.67-1.60 (m, 2H), 1.40-1.27 (m, 2H) ppm **^{13}C NMR** (100 MHz $CDCl_3$) δ 140.2, 125.7, 123.0, 120.5, 118.8, 108.6, 61.7, 57.0, 43.0, 39.2, 31.5, 30.7, 25.6, 24.4 ppm. **LRMS** m/z (ES+) m/z: 293 $[M+H]^+$. **HRMS** (ESI) m/z calcd. for $C_{20}H_{24}N_2$ [M +H], 293.19 found 293.2014.

1-(2-(1-Methylpiperidin-2-yl)ethyl)-1H-benzo[d]imidazole (13d): Yield: 70%. **^{1}H NMR** (400 MHz CD_3OD) δ 8.86 (s, 1H), 8.33-8.22 (m, 2H), 7.99-7.90 (m, 2H), 5.03-4.95 (m, 2H), 3.55-3.50 (m, 1H), 2.90 (s, 3H), 2.70-2.42 (m, 4H), 2.28-2.24 (m, 2H), 2.00-1.80 (m, 4H) ppm. **^{13}C NMR** (100 MHz CD_3OD) δ 144.0, 143.3, 134.0, 123.7, 122.9, 119.6, 110.7, 62.2, 57.1, 42.1, 32.8, 30.6, 30.1, 25.4, 24.1 ppm. **LRMS** m/z (ES+) m/z: 244 $[M+H]^+$. **HRMS** (ESI) m/z calcd. for $C_{15}H_{22}N_3$ [M +H] 244.1808, found 244.1811.

Synthesis of 1-(3-chloropropyl)-1H-indole (15)

Indole (0.52 mmol, 1 eq.) was added to 10 mL of DMF in a double neck round bottomed flask. NaH (0.57 mmol, 1.1 eq.) was added to the stirring solution at 0 °C, and the mixture was allowed to reach r.t stirring for 20 minutes. Then, 1-bromo-3-chloropropane (0.57 mmol 1.1 eq.) was added. The reaction mixture was allowed to stir under N_2 atmosphere for 12h at r.t. The reaction was quenched with water (10 mL) and extracted twice with EtOAc (20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure giving a yellow-brown crude oil. The crude product (**15**) was purified by chromatography on silica gel, using hexane/EtOAc (4:1) as eluent. **Yield:** 82% **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.69 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.27 (t, *J* = 8.0 Hz, 1H), 7.19-7.15 (m, 2H), 6.56 (d, *J* = 4.0 Hz, 1H), 4.37-4.33 (m, 2H), 3.46 (t, *J* = 8.0 Hz, 1H), 3.32 (t, *J* = 8.0 Hz, 1H), 2.37-2.26 (m, 2H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 135.9, 128.8, 128.1, 121.8, 121.2, 119.6, 109.4, 101.6, 42.9, 42.0, 32.7 ppm. **LRMS** m/z (ES+) m/z: 194 $[M+H]^+$

Synthesis of 1-(3-(4-methylpiperazin-1-yl)propyl)-1H-indole (16).

The derivative 16 was synthesised following the procedure used for the synthesis of compounds **7a-i**. **Yield:** 63%. **^{1}H NMR** (400 MHz $CDCl_3$) δ 7.51-7.47 (m, 2H), 7.40 (d, *J* =

8.0 Hz, 1H), 7.30-7.24 (m, 1H), 7.19 (t, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 12.0 Hz, 2H), 3.24-3.23 (d, *J* = 8.0 Hz, 2H), 2.47 (s, 3H), 2.35 (m, 4H), 1.46-1.41 (m, 4H), 1.32-1.29 (m, 2H) ppm. **^{13}C NMR** (100 MHz $CDCl_3$) δ 163.7, 138.9, 135.8, 135.7, 132.3, 132.2, 126.7, 126.7, 122.2, 118.0, 116.2, 56.8, 56.1, 25.2, 24.2, 16.0 ppm. **LRMS** m/z (ES+) m/z: 258 $[M+H]^+$ **HRMS** (ESI) m/z calcd. for $C_{16}H_{24}N_3$ [M +H] 258.1965, found 258.1966.

4.2 Biology

Bacterial strains and growth conditions: The bacterial species used in this study were *M. smegmatis* mc2155 (ATCC 700084), *M. bovis* BCG Pasteur (ATCC 35734), *M. tuberculosis* mc27000, *M. tuberculosis* H37Rv (ATTC27294), *M. tuberculosis* CF73 and two MDR-TB clinical isolates (CF104 and CF81) obtained from. Mycobacterial species were cultured in either Middlebrook 7H9 broth or Middlebrook 7H10 agar media supplemented with albumin-dextrose-catalase (ADC) or oleic acid-albumin-dextrose-catalase (OADC) enrichments, respectively, purchased from BD Biosciences. All reagents were purchased from Sigma-Aldrich unless stated otherwise.

Bacterial growth inhibition assays.

The MIC of the compounds against *M. smegmatis* mc2155, *M. bovis* BCG, and *M. tuberculosis* mc27000 were calculated by standard MABA (Microplate Alamar Blue assay) as previously described.[27] Briefly, 200 µL of sterile deionized water was added to all outer-perimeter wells of a sterile 96-well plate (Corning Incorporated, Corning, NY) to minimize evaporation of the medium in the test wells during incubation. The wells in rows B to G in columns 3 to 11 received 100 µL of 7H9 medium containing 0.2% casamino acids, 24 µg/mL pantothenate and 10% OADC (Beckton Dickinson, Sparks, MD). Compounds were added to rows B–G followed by 1:2 serial dilutions across the plate to column 10, and 100 µL of excess medium was discarded from the wells in column 10. The bacterial culture at 0.5 McFarland standard diluted 1:50 (100 µL) was added to the wells in rows B to G in columns 2 to 11, where the wells in column 11 served as drug-free controls. The plates were sealed with parafilmTM and were incubated at 37 °C. A freshly prepared 1:1 mixture of Alamar Blue (Celltiter-Blue™, Promega Corp, Madison, WI) reagent and 10% Tween® 80 (50 µL) and re-incubated at 37 °C for 24 h.

Determination of Minimal Inhibitory Concentration (MIC_{90})

The anti-*M. tuberculosis* activity of the compounds against *M. tuberculosis* H37Rv (ATTC27294), *M. tuberculosis* CF73 and two MDR-TB clinical isolates (CF104 and CF81) was determined using the Resazurin Microtiter Assay (REMA) method according to Palomino *et al.*, [29]. Stock solutions of the tested compounds were prepared in dimethyl sulfoxide (DMSO), then diluted in Middlebrook 7H9 broth (Difco, Detroit, MI, USA) supplemented with oleic acid, albumin, dextrose and catalase (OADC enrichment) to obtain a final drug concentration range of 0.09–100 µg/mL. A suspension of the *M.*

tuberculosis $H_{37}Rv$ ATCC 27294 and clinical isolates were cultured in Middlebrook 7H9 broth supplemented with OADC and 0.05% Tween 80 for one week at 37 °C in an atmosphere of 5% CO_2.. The concentration was adjusted at McFarland 1 and diluted to 2.4 × 10^5 CFU/mL. 100 μL of the inoculum was added to each well of a 96-well microplate together with 100 μL of the compounds. The plate was incubated for 7 days at 37 °C in an atmosphere of 5% CO_2. After 24 h, 30 μL 0.01% resazurin (solubilized in water) was added. The fluorescence of the wells was read after 24 h using a Cytation 3 (BioTek®, Winooski, VT, USA). The MIC_{90} was defined as the lowest concentration resulting in 90% inhibition of growth of *M. tuberculosis*. Samples were set up in three independent assays.

Cytotoxic Analysis (IC_{50}) of MRC-5 cell line

In these experiment, cells were collected in a solution of trypsin/ethylenediamine tetracetic acid (EDTA) (Vitrocell®) and centrifuged (252× *g* for 5 min). The number of cells was counted using a Neubauer chamber (Celeromics, Valencia, Spain) after staining non-viable cells with 0.4% trypan blue solution (Sigma-Aldrich®) via the cell exclusion assay. Then, the cell concentration was adjusted to 7.5 × 10^4 cells/mL in DMEM for tumor cells and MRC-5 cells (ATCC CCL-171). Next, a 200 μL suspension was deposited into each well of a 96-well microplate to a cell density of 1.5 × 10^4 cells/well. The cells were incubated at 37 °C in an atmosphere of 5% CO_2 for 24 h to allow the cells to attach to the plate [30]. The compounds were solubilized in DMSO to an initial concentration of 10,000 μg/mL. Test solutions of the compounds were prepared to obtain concentrations from 500 to 1.95 μg/mL. The diluted solutions were added to the cells after changing the medium to remove any non-adherent cells, and the cultures were incubated for an additional 24 h. The cytotoxicity of the compounds was determined after incubating the cells in 30 μL of resazurin for approximately 2 h. The measurement was performed using a Synergy H1 microplate reader (BioTek®, Winooski, VT, USA) with excitation and emission filters at wavelengths of 530 and 590 nm, respectively. The assays were performed in three independent experiments.

Efflux pump inhibition assays

The assay was performed based on previously published protocols.[26] In brief, early log phase cells of *M. smegmatis* were taken and the OD_{600} was adjusted to 0.4 in 1× PBS. The test samples contained (4−6) × 10^7 bacteria/mL in PBS, 0.4% glucose (as a source of energy for efflux pumps activity), 0.5 mg/L ethidium bromide (as a substrate for efflux pumps), and the compounds being tested at 1/4× MIC concentrations. Blank samples contained all of the components mentioned above, except the bacterial suspension, which was replaced with 1× PBS. Verapamil and chlorpromazine, known efflux pump inhibitors, were used as positive controls at concentrations of 125 μg/mL and xx respectively. The experiment was performed in a 96-well plates that was read in a fluorimeter (FLUOstar OPTIMA, BMG Labtech) with the following parameters: wavelengths of 544 and 590 nm for excitation and detection of fluorescence, gain 2200, a temperature of 37 °C, and a cycle of measurement every minute for a total period of 60 min. The accumulation or efflux of ethidium bromide was monitored in real-time for the mentioned period.

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REFERENCES

1. http://www.who.int/tb/publications/global_report/en/

2. E.W. Tiemersma, M.J. van der Werf, M.W. Borgdorff, B.G. Williams, N.J.D. Nagelkerke, Natural history of tuberculosis: Duration and fatality of untreated pulmonary tuberculosis in HIV negative patients: A systematic review, PLoS One 6 (2011) e17601.

3. C. Geldmacher, A. Zumla, M. Hoelscher, Interaction between HIV and Mycobacterium tuberculosis: HIV-1-induced CD4 T-cell depletion and the development of active tuberculosis, Curr. Opin. HIV AIDS 7 (2012) 268–274.

4. R.D. Deshmukh, D.J. Dhande, K.S. Sachdeva, A. Sreenivas, A.M. V Kumar, S. Satyanarayana, M. Parmar, P.K. Moonan, T.Q. Lo, Patient and provider reported reasons for lost to follow up in MDRTB treatment: A qualitative study from a drug resistant TB Centre in India, PLoS One 10 (2015) e0135802.

5. J.A. Caminero, Treatment of multidrug-resistant tuberculosis:evidence and controversies, Int. J. Tuberc. Lung Dis. 10 (2006) 829−837.

6. E.D. Chan, V. Laurel, M.J. Strand, J. F. Chan, M. L. Huynh, M. Goble, M.D. Iseman, Treatment and outcome analysis of 205 patients with multidrug-resistant tuberculosis, Am. J. Respir. Crit. Care Med. 169 (2004) 1103−1109.

7. B. Eker, J. Ortmann, G. B. Migliori, G. Sotgiu, R. Muetterlein, R. Centis, H. Hoffmann, D. Kirsten, T. Schaberg, S. Ruesch-Gerdes, C. Lange, Multidrug- and extensively drug-resistant tuberculosis, Emerging Infect. Dis. 14 (2008) 1700−1706.

8. C.D. Mitnick, S.S. Shin, K.J. Seung, M. L. Rich, S.S. Atwood, J.J. Furin, G.M. Fitzmaurice, F.A. Alcantara Viru, S.C. Appleton, J.N. Bayona, C.A. Bonilla, K. Chalco, S. Choi, M.F. Franke, H. S. Fraser, D. Guerra, R. M. Hurtado, D. Jazayeri, K. Joseph, K. Llaro, L. Mestanza, J.S. Mukherjee, M. Muñoz, E. Palacios, E. Sanchez, A. Sloutsky, M.C. Becerra, Comprehensive treatment of extensively drug-resistant tuberculosis, N. Engl. J. Med. 359 (2008) 563−574.

9. A. A. Velayati, P. Farnia, M.R. Masjedi, The totally drug resistant tuberculosis (TDR-TB), Int. J. Clin. Exp. Med. 6 (2013) 307−309.

10. K. Hards, J.R. Robson, M. Berney, L. Shaw, D. Bald, A. Koul, K. Andries, G.M. Cook, Bactericidal mode of action of bedaquiline, J. Antimicrob. Chemother. 70 (2014) 2028–2037.

11. B. Chan, T.M. Khadem, J. Brown, A review of tuberculosis: Focus on bedaquiline, Am. J. Heal. Pharm. 70 (2013) 1984–1994.

12. M. Protopopova, C. Hanrahan, B. Nikonenko, R. Samala, P. Chen, J. Gearhart, L. Einck, C.A. Nacy, Identification of a new antitubercular drug candidate, SQ109, from a combinatorial library of 1,2-ethylenediamines, J. Antimicrob. Chemother. 56 (2005) 968−974.

13. Q. Ruan, Q. Liu, F. Sun, L. Shao, J. Jin, S. Yu, J. Ai, B. Zhang, W. Zhang, Emerg Microbes Infect. 5 (2016) e12.

14. M. Lee, J. Lee, M.W. Carroll, H. Choi, S. Min, T. Song, L.E. Via, L.C. Goldfeder, E. Kang, B. Jin, H. Park, H. Kwak, H. Kim, H. S. Jeon, I. Jeong, J.S. Joh, R.Y. Chen, K.N. Olivier, P.A. Shaw, D. Follmann, S.D. Song, J.K. Lee, D. Lee, C.T. Kim, V. Dartois, S.K. Park, S.N. Cho, C.E. Barry III, N. Engl. J. Med. 367 (2012) 1508-18.

15. J.C. Johnston, N.C. Shahidi, M. Sadatsafavi, J.M. Fitzgerald, Treatment outcomes of multidrug-resistant tuberculosis: A systematic review and meta-analysis, PLoS One. 4 (2009) 0006914.

16. L. Amaral, M. Viveiros, J.E. Kristiansen, "Non-Antibiotics": alternative therapy for the management of MDRTB and MRSA in economically disadvantaged countries, Curr. Drug Targets. 7 (2006) 887–891.

17. O.Y. Limanskaya, T.N. Mukhina, V.N. Stepanshina, I.G. Shemyakin, X. Wu, J. Zhang, T. V Fesenko, V.A. Pokrovskiy, A.P. Limanskii, Identification of wild-type Mycobacterium tuberculosis isolates and point mutations associated with isoniazid resistance, Mol. Biol. 44 (2010) 559–567.

18. L. Amaral, J.E. Kristiansen, M. Viveiros, J. Atouguia, Activity of phenothiazines against antibiotic-resistant Mycobacterium tuberculosis: a review supporting further studies that may elucidate the potential use of thioridazine as anti-tuberculosis therapy, J. Antimicrob. Chemother. 47 (2001) 505–511.

19. L. Amaral, J. Molnar, Why and How the Old Neuroleptic Thioridazine Cures the XDR-TB Patient, Pharmaceuticals (Basel), 5 (2012) 1021–1031.

20. L. Amaral, J. Molnar, Potential therapy of multidrug-resistant and extremely drug-resistant tuberculosis with thioridazine, In Vivo (Brooklyn) 26 (2012) 231–236.

21. L. Rodrigues, J. Ramos, I. Couto, L. Amaral, M. Viveiros, Ethidium bromide transport across Mycobacterium smegmatis cell-wall: correlation with antibiotic resistance, BMC Microbiol. 11 (2011) 35.

22. D. Machado, I. Couto, J. Perdigo, L. Rodrigues, I. Portugal, P. Baptista, B. Veigas, L. Amaral, M. Viveiros, Contribution of efflux to the emergence of isoniazid and

multidrug resistance in Mycobacterium tuberculosis, PLoS One. 7 (2012). 0034538.

23. J. de Keijzer, A. Mulder, P.E.W. de Haas, A.H. de Ru, E.M. Heerkens, L. Amaral, D. van Soolingen, P.A. van Veelen, Thioridazine alters the cell-envelope permeability of *Mycobacterium tuberculosis*, J. Proteome Res. 15 (2016) 1776–1786

24. M. Martins, Z. Schelz, A. Martins, J. Molnar, G. Hajs, Z. Riedl, M. Viveiros, I. Yalcin, E. Aki-Sener, L. Amaral, In vitro and ex vivo activity of thioridazine derivatives against Mycobacterium tuberculosis, Int. J. Antimicrob. Agents. 29 (2007) 338–340.

25. F. Schlauderer, K. Lammens, D. Nagel, M. Vincendeau, A.C. Eitelhuber, S.H.L. Verhelst, D. Kling, A. Chrusciel, J. Ruland, D. Krappmann, K.-P. Hopfner, Structural analysis of phenothiazine derivatives as allosteric inhibitors of the MALT1 paracaspase, Angew. Chem. Int. Ed. 52 (2013) 10384–10387.

26. The N-methylated indole analogues of **12e** were previously shown to be not active against MTB. M. Pieroni, D. Machado, E. Azzali, S. Santos Costa, I. Couto, G. Costantino, M. Viveiros, Rational Design and Synthesis of Thioridazine Analogues as Enhancers of the Antituberculosis Therapy, J. Med. Chem. 58 (2015) 5842-5853.

27. S. Bhakta, N. Scalacci, A. Maitra, A.K. Brown, S. Dasugari, D. Evangelopoulos, T.D. McHugh, P.N. Mortazavi, A. Twist, E. Petricci, F. Manetti, D. Castagnolo, Design and Synthesis of 1-((1,5-bis(4-chlorophenyl)-2-methyl-1H-pyrrol-3-yl)methyl)-4-methylpiperazine (BM212) and N-adamantan-2-yl-N'-((E)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine (SQ109) pyrrole hybrid derivatives: discovery of potent antitubercular agents effective against multidrug-resistant mycobacteria, J. Med. Chem. 59 (2016) 2780-93.

28. E. Pelizzetti, E. Mentasti, Cation radicals of phenothiazines. Electron transfer with aquoiron(II) and -(III) and hexacyanoferrate(II) and -(III), Inorg. Chem. 18 (1979) 583–588.

29. J. Palomino, A. Martin, M. Camacho, H. Guerra, J. Swings, F. Portaels, Resazurin microtiter assay Plate: simple and inexpensive method for detection of drug resistance in Mycobacterium tuberculosis, Antimicrob. Agents Chemother. 46 (2002) 2720–2722.

30. J. O´Brien, I. Wilson, T. Orton, F. Pognan, Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity, Eur. J. Biochem. 267 (2000) 5421-5426.