Synthesis and evaluation of antitubercular agents 2-aminothiophenes and benzo-1,2-selenazol-3(2*H*)-ones targeting Pks13 and Ag85C respectively

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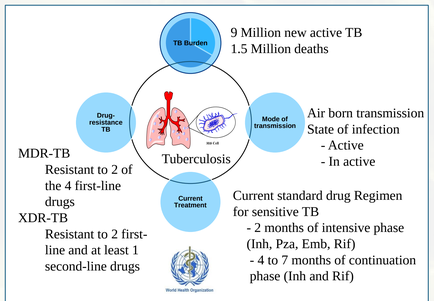
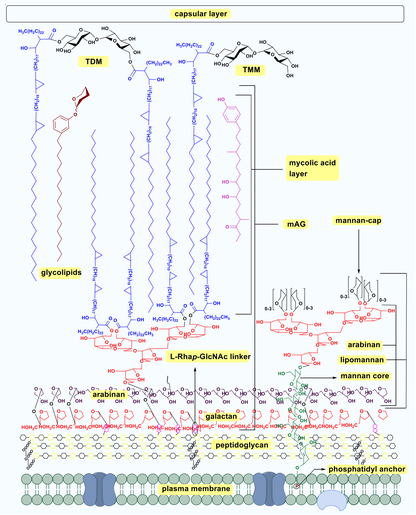
Tuberculosis[1]

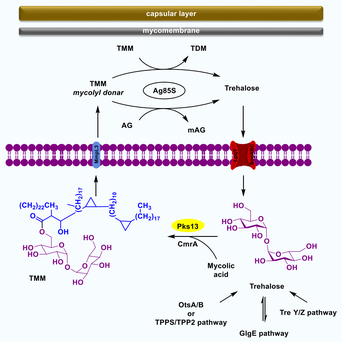


Mycobacterium tuberculosis (*Mtb*) is the causative agent of tuberculosis (TB). TB ranks first for the number of deaths in humans caused by an infectious agent. This fact emphasizes the need to identify new drugs to treat TB. *Mtb* cell wall: The cell wall of *Mtb* is a bastion (Fig. 1). It is composed of a plasma membrane, a peptidoglycan layer, a layer of extractable, non-covalently linked glycans with mycolylarabinogalactan (mAG), glycolipids and an outer capsule.

Figure 1: *Mtb* Cell wall[2]



Scheme 1: Trehalose Utlizaiton Pathway (TUP)



TUP: The TUP contain a number of genetically valid potential drug targets such as OtsA/B, GlgE, Pks13, MmpL3 and the Ag85 complex. Inhibiting one or more of these enzymes may lead to novel drugs which can cure active, latent, and MDR-TB.

The enzyme Pks13 catalyzes the condensation of trehalose with mycolic acid to produce trehalose monomycolate (TMM). TMM is utilized by the Ag85 complex (Ag85s), a homologous family of enzymes (Ag85 A, B and C) that catalyzes a transesterification reaction that transfers mycolic acids (MAs) onto arabinogalactan (AG), trehalose and TMM forming mycolylarabinogalactan (mAG), TMM, and trehalose dimycolate (TDM, Cord Factor), respectively.

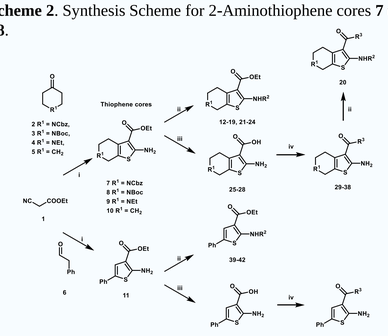
Synthesis and Microbiological Evaluation of 2-Amino-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridines Against Sensitive and Drug resistant *Mtb*.[3]

TB and its drug resistant forms kills more people than any other infectious disease. This fact emphasizes the need to identify new drugs to treat TB. 2-Aminothiophenes (2AT) have been reported to inhibit Pks13, a validated anti-TB drug target. We synthesized a library of 42 2AT compounds. Among these, compound **18** showed remarkable potency against *Mtb* H37RV (MIC = 0.23 μM) and showed an impressive potency (MIC = 0.20–0.44 μM) against *Mtb* strains resistant to isoniazid, rifampicin and fluoroquinolones. The site of action for the compound **18** is presumed to be Pks13 or an earlier enzyme in the mycolic acid biosynthetic pathway. This inference is based on structural similarity of the compound **18** with known Pks13 inhibitors, which is corroborated by mycolic acid biosynthesis studies showing that the compound strongly inhibits the biosynthesis of all forms of mycolic acid in *Mtb*. In summary, these studies suggest **18** represents a promising anti-TB lead that exhibits activity well below toxicity to human monocytic cells.

2-Aminothiophens 1st Generation

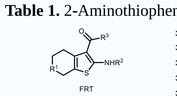
Synthesis

Scheme 2. Synthesis Scheme for 2-Aminothiophene cores **7** to **48**.



Reagents and Conditions: (i) S_8, Et_2NH, EtOH, **7** (86%), **8** (63%), **9** (75%), **10** (83%), **11** (61%); (ii) Acid chloride (AC), Et_3N, DCM, DMAP (15–82%); (iii) LiOH, THF : H_2O: MeOH (3 : 1 : 1) (44–89%); (iv) Alkylamine (AA), EDC. HCl, HOBt, DMAP, dry CH_2Cl_2 (21–61%).

Table 1. 2-Aminothiophene Compound Library.



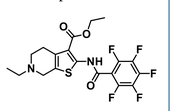
12, 41%; R1 = NBoc, R2 = CH2CO, R3 = OEt
13, 23%; R1 = NBoc, R2 = C6H5CO, R3 = OEt
14, 82%; R1 = NBoc, R2 = C6F5CO, R3 = OEt
15, 39%; R1 = NBoc, R2 = 3,5-(CF3)2C6H3CO, R3 = OEt
16, 45%; R1 = NEt, R2 = CH2CO, R3 = OEt
17, 35%; R1 = NEt, R2 = C6H5CO, R3 = OEt
18, 45%; R1 = NEt, R2 = C6F5CO, R3 = OEt
19, 43%; R1 = NEt, R2 = 3,5-(CF3)2C6H3CO, R3 = OEt
20, 22%; R1 = NEt, R2 = 3,5-(CF3)2C6H3CO, R3 = 4-(CH3)BnNH
21, 59%; R1 = CH2, R2 = CH2CO, R3 = OEt
22, 57%; R1 = CH2, R2 = C6H5CO, R3 = OEt
23, 50%; R1 = CH2, R2 = C6F5CO, R3 = OEt
24, 15%; R1 = CH2, R2 = 3,5-(CF3)2C6H3CO, R3 = OEt
25, 69%; R1 = NCbz, R2 = H, R3 = OH
26, 89%; R1 = NBoc, R2 = H, R3 = OH
27, 66%; R1 = NEt, R2 = H, R3 = OH
28, 44%; R1 = CH2, R2 = H, R3 = OH
29, 36%; R1 = NEt, R2 = H, R3 = BuNH
30, 25%; R1 = NEt, R2 = H, R3 = BnNH
31, 40%; R1 = NEt, R2 = H, R3 = 4-(CH3)BnNH
32, 30%; R1 = NEt, R2 = H, R3 = 2-(OCH3)BnNH
33, 29%; R1 = NEt, R2 = H, R3 = AdamantylNH
34, 26%; R1 = CH2, R2 = H, R3 = BuNH
35, 24%; R1 = CH2, R2 = H, R3 = BnNH
36, 36%; R1 = CH2, R2 = H, R3 = 4-(CH3)BnNH
37, 23%; R1 = CH2, R2 = H, R3 = 2-(OCH3)BnNH
38, 21%; R1 = CH2, R2 = H, R3 = AdamantylNH

39, 58%; R2 = CH2CO, R3 = OEt
40, 94%; R2 = C6H5CO, R3 = OEt
41, 45%; R2 = C6F5CO, R3 = OEt
42, 45%; R2 = 3,5-(CF3)2C6H3CO, R3 = OEt
43, 83%; R2 = H, R3 = OH
44, 61%; R2 = H, R3 = BuNH
45, 59%; R2 = H, R3 = BnNH
46, 57%; R2 = H, R3 = 4-(CH3)BnNH
47, 68%; R2 = H, R3 = 4-(CH3)BnNH
48, 44%; R2 = H, R3 = AdamantylNH

Inhibition Studies

Ag85C Inhibition Assay: No inhibition observed. **MIC test**: Compounds showed MIC in the range of 0.23 to > 600 μM. Compound **18** showed MIC of 0.23 μM

Compound **18**



18

Mtb H37Rv, MIC = 0.23 mM

Table 2. Evaluation of **18** MICs and ICs (μM) Against *Mtb* under aerobic, Low oxygen, and Intracellular conditions. Evaluation of **18** Against Drug resistant strains of *Mtb*

H37Rv (aerobic)	H37Rv (low oxygen)	H37Rv (intracellular)	INH-R1	INH-R2	RIF-R1	RIF-R2	FQ-R1
MIC = 0.23	MIC > 200		MIC = 0.44	MIC = 0.20	MIC = 0.30	MIC = 0.44	MIC = 0.37
MIC_{50} = 0.12	IC_{50} = 0.27	IC_{50} = 5.6	IC_{50} = 0.16	IC_{50} = 0.053	IC_{50}= 0.095	IC_{50} = 0.11	IC_{50} = 0.14
MIC_{90} = 0.23	IC_{90} = 15	IC_{90} = 9.75	IC_{90} = 0.45	IC_{90} = 0.20	IC_{90} = 0.35	IC_{90} = 0.48	IC_{90} = 0.39
Controls							
RIF	MIC = 0.048, n=8		MIC = 0.012	MIC = 0.0070	MIC = 3.5	MIC > 50	MIC = 0.020
INH		IC_{50} = 0.24	MIC > 200	MIC > 200	MIC = 0.18	MIC > 0.60	MIC = 0.32
Levofloxacin			MIC = 1.3	MIC = 1.4	MIC = 1.2	MIC = 1.2	MIC = 24

Inhibition of Mycolic Acid Biosynthesis

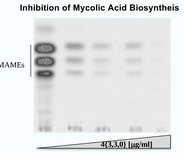
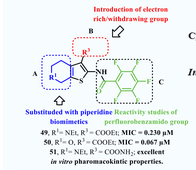


Figure 2. Effect of **18** on mycolic acid synthesis. TLC analysis: Mobile phase; $CHCl_3$: MeOH : H_2O (20 : 4 : 0.5 by vol)

2-Aminothiophens 2nd Generation



Pk properties of Compound **51**

Cytochrome P450 inhibition	6 Cytochrome P450 enzyme isoforms IC_{50} (μM)	> 20
***In vitro* microsomal stability**	NADPH-dependent CL_{int} (μL/min/mg)	< 12.8
	NADPH-Free CL_{int} (μL/min/mg)	< 12.8
	NADPH-dependent $T_{1/2}$ (min)	> 180
	NADPH-Free $T_{1/2}$ (min)	> 180
HepG2 Cytotoxicity	IC_{50} (μM)	> 100

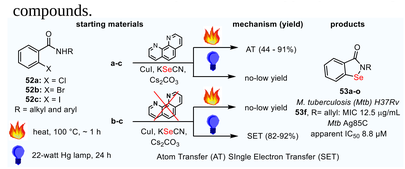
In vitro pharmacokinetic (Pk) properties of **51**: No-Cytotoxicity, Excellent microsomal stability (Intrinsic clearance (CL_{int}) of < 12 μL/min/mg and Half life ($T_{1/2}$) > 180 mins). The compounds **51** did not inhibit any CYP450 isoforms tested below IC_{50} = 20 μM minimizing the risk of drug-drug interaction.

Thermal and Photoinduced Cu-promoted C-Se bond formation: Synthesis of 2-Alkyl-1,2-benzisoselenazol-3(2*H*)-ones and Evaluation against *Mtb*.[4, 5]

2-Alkyl-1,2-benzisoselenazol-3(2*H*)-ones, represented by the ebselen (**53a**), are being studied intensively for a range of medicinal applications. We describe both a new thermal and photoinduced copper-mediated cross-coupling between potassium selenocyanate (KSeCN) and N-substituted *ortho*-halobenzamides to form 2-alkyl-1,2-benzisoselenazol-3(2*H*)-ones containing a C-Se-N bond. The copper ligand (1,10-phenanthroline) facilitates C-Se bond formation during heating via a mechanism that likely involves atom transfer (AT); whereas, in the absence of ligand, photoinduced activation likely proceeds through a single electron transfer (SET) mechanism. A library of fifteen 2-alkyl-1,2-benzisoselenazol-3(2*H*)-ones was prepared. One member of the library was azide-containing derivative **53j** that was competent to undergo a strain-promoted azide-alkyne cycloaddition. The library was evaluated for inhibition of *Mtb* growth and *Mtb* Antigen 85C (*Mtb* Ag85C) activity. Compound **53f** was most potent with a minimal inhibitory concentration (MIC) of 12.5 μg/mL and an *Mtb* Ag85C apparent IC_{50} of 8.8 μM.

Synthesis

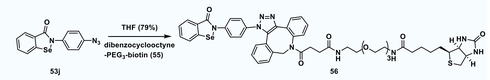
Scheme 3. Thermal and photoinduced copper-promoted sythesis of 2-alkyl-1,2-benzisoselenazol-3(2*H*)-ones compounds.



Reactions and Conditions: i) Thermal activation: 1.0 equiv phen-CuI, 1.2 equiv KSeCN, 2.5 equiv Cs_2CO_3, 0.4-1.5 h 100-110 °C; ii) Photochemical activation: catalyst, hυ, 1.2 equiv base, 2.5 equiv KSeCN, CH_3CN, 0-20 °C, 24 h

Table 3. Thermal and Photoinduced Synthesis of a 1,2-Benzisoselenazol-3(2*H*)-one Library

entry	substrate	R	product, yield (%)	entry	substrate	R	product, yield (%)	entry	substrate	R	product, yield (%)
1	52c		53a, 91	6	54f		53f, 39	11	54k		53k, 75
2	54b		53b, 63	7	54g		53g, 63	12	54l		53l, 72
3	54c		53c, 44	8	54h		53h, 52	13	54m		53m, 78
4	54d		53d, 67	9	54i		53i, 35	14	54n		53n, 85
5	54e		53e, 39	10	54j		53j, 23	15	54o		53o, 85



Scheme 4. Proposed Cu-promoted Mechanisms

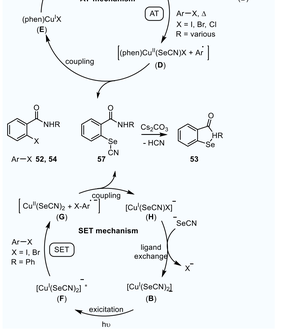


Table 4. Activity of 2-Alkyl-1,2-benzisoselenazol-3(2*H*)-ones against *Mtb* H_{37}Rv and *Mtb* Ag85C

Compound No.	cLogP	MIC (μg/ml)	bMtb Ag85C activity (%)	Ag85C apIC50 (μM)	Compound No.	cLogP	MIC (μg/ml)	bMtb Ag85C activity (%)	Ag85C apIC50 (μM)	Compound No.	cLogP	MIC (μg/ml)	bMtb Ag85C activity (%)	Ag85C apIC50 (μM)
53a	3.70	12.5	17 ± 3	5.12	**53g**	4.60	50	30 ± 16	5.3	**53m**	3.43	25	40 ± 17	4.1
53b	3.65	25	24 ± 17	0.54	**53h**	3.85	25	19 ± 2	0.72	**53n**	3.99	25	44 ± 15	3.7
53c	3.65	12.5	36 ± 4	28.6	**53i**	3.85	25	21 ± 7	1.0	**58**	5.76	100	80 ± 7	> 100
53d	3.62	25	20 ± 4	1.2	**53j**	4.15	25	20 ± 4	1.1	**56**	5.26	N/A	61 ± 11	2.02
53e	3.73	25	20 ± 10	6.5	**53k**	3.20	25	31 ± 12	1.5					
53f	2.73	12.5	15 ± 2.0	8.8	**53l**	4.62	50	59 ± 14	25					

Conclusion

1)1st Gen. 2AT: i. Compound **18** with remarkable potency against *Mtb* H37RV (MIC = 0.23 μM) and showed an impressive potency (MIC = 0.20–0.44 μM) against *Mtb* strains resistant to isoniazid, rifampicin and fluoroquinolones; 2) 2nd Gen. 2AT: i. Compound **50** MIC = 0.067 μM. ii. Compound **51** non-cytotoxic excellent invitro Pk properties; 3) i. Developed thermal and photoinduced Cu-promoted C-Se coupling reaction (first photoinduced copper-mediated C-Se cross-coupling). iii. Synthesized 2-alkyl-1,2-benzisoselenazol-3(2*H*)-ones compounds with excellent anit-*Mtb* activity.

References

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