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Appendices

Details of interacting residues in tabular form

Table A-1. Interacting residues and amino acids of identified compounds against *MmpL* 11D2.

1. CSID1653545

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	426A	GLN	3.44	733	22
2	428A	LEU	3.46	732	42
3	474A	LEU	3.30	733	457
4	496A	ARG	3.99	739	580
5	500A	PRO	3.80	745	627
6	503A	ALA	3.50	750	658
7	508A	VAL	3.51	750	693
8	509A	ASP	3.07	729	701

Hydrogen Bonds ——

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	496A	ARG	1.85	2.58	140.76	✓	✓	582 [Ng+]	722 [O2]
2	496A	ARG	3.11	3.59	117.93	✓	✓	585 [Ng+]	722 [O2]
3	509A	ASP	2.04	2.95	161.13	✓	✓	703 [O3]	719 [Nam]
4	509A	ASP	2.49	2.95	110.50	×	✓	719 [Nam]	703 [O3]
5	510A	VAL	2.00	2.83	162.36	✓	×	706 [Nam]	722 [O2]

2. CSID1655442

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	496A	ARG	3.42	724	580
2	500A	PRO	3.06	726	627
3	508A	VAL	3.19	726	694
4	510A	VAL	3.51	724	712

Hydrogen Bonds ——

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	496A	ARG	3.12	3.81	138.29	✓	✓	582 [Ng+]	737 [Nar]
2	508A	VAL	1.90	2.72	160.29	✓	×	689 [Nam]	722 [O2]
3	509A	ASP	2.77	3.43	128.04	✓	✓	703 [O3]	737 [Nar]
4	510A	VAL	2.15	2.93	150.32	✓	×	706 [Nam]	737 [Nar]

3. CSID1438694

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	496A	ARG	2.98	722	580
2	500A	PRO	3.39	720	627
3	507A	GLN	3.10	748	682
4	508A	VAL	3.29	720	694
5	508A	VAL	4.00	719	693
6	510A	VAL	3.35	722	712

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	508A	VAL	2.45	3.24	152.88	✔	✖	689 [Nam]	738 [Nam]
2	508A	VAL	2.39	3.05	127.57	✖	✖	738 [Nam]	692 [O2]
3	510A	VAL	1.76	2.57	155.20	✔	✖	706 [Nam]	727 [O2]

4. CSID2153432

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	500A	PRO	3.27	736	626
2	500A	PRO	3.29	742	627
3	503A	ALA	3.59	738	658
4	508A	VAL	3.78	738	695
5	508A	VAL	3.80	742	693
6	508A	VAL	3.31	743	694
7	509A	ASP	3.65	753	701
8	510A	VAL	3.34	744	712

✔ Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	508A	VAL	2.11	2.86	145.01	✔	✖	689 [Nam]	719 [O2]

5. CSID930923

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	426A	GLN	3.66	731	22
2	428A	LEU	3.38	731	42
3	474A	LEU	3.96	731	457
4	496A	ARG	3.61	740	580
5	509A	ASP	3.14	725	701

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	496A	ARG	1.85	2.55	136.95	✓	✓	582 [Ng+]	738 [O2]
2	496A	ARG	3.24	3.69	115.23	✓	✓	585 [Ng+]	738 [O2]
3	509A	ASP	1.88	2.75	153.15	✓	✓	703 [O3]	735 [Nam]
4	509A	ASP	2.12	2.75	124.45	×	✓	735 [Nam]	703 [O3]
5	510A	VAL	2.09	2.93	167.04	✓	×	706 [Nam]	738 [O2]

6. DB12983

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	445A	ILE	3.13	748	195
2	463A	PRO	3.18	758	358
3	464A	PRO	3.22	746	366
4	465A	ARG	3.99	753	373
5	466A	PHE	3.77	751	389

7. DB15039

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	496A	ARG	3.94	739	580
2	496A	ARG	3.44	740	579
3	500A	PRO	3.37	755	626
4	500A	PRO	3.03	742	627
5	503A	ALA	3.84	757	658
6	508A	VAL	3.02	741	694
7	510A	VAL	3.61	741	712

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	496A	ARG	2.26	2.98	140.80	✔	✔	582 [Ng+]	719 [O2]
2	496A	ARG	2.74	3.35	129.38	✔	✔	585 [Ng+]	719 [O2]
3	508A	VAL	2.52	3.27	146.48	✔	✖	689 [Nam]	752 [Nar]
4	510A	VAL	2.09	2.80	138.93	✔	✖	706	719 [O2]

8. DB14785

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	496A	ARG	3.97	722	579
2	500A	PRO	3.43	747	627
3	507A	GLN	3.70	739	682
4	508A	VAL	3.49	747	694

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	493A	THR	2.06	2.85	158.65	✔	✔	546 [O3]	766 [O3]
2	508A	VAL	1.83	2.63	155.60	✔	✖	689 [Nam]	727 [O2]
3	508A	VAL	1.98	2.84	146.40	✖	✖	749 [O3]	692 [O2]

9. DB15688

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	440A	GLU	3.66	752	141
2	440A	GLU	3.91	763	140
3	447A	ALA	3.27	723	207
4	501A	ARG	3.37	738	633
5	502A	VAL	3.58	733	651
6	502A	VAL	3.35	737	652

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	443A	GLN	2.75	3.22	115.72	✔	✖	167 [Nam]	742 [O2]
2	444A	THR	2.07	2.77	137.83	✔	✖	179 [Nam]	742 [O2]
3	444A	THR	2.05	2.92	142.93	✖	✔	722 [Nam]	184 [O3]
4	501A	ARG	2.20	2.77	113.32	✖	✖	731 [Nam]	632 [O2]

10. ZINC000051951669

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	431A	PHE	3.33	752	80
2	431A	PHE	3.10	751	78
3	432A	ASP	3.36	758	88
4	444A	THR	3.65	754	185
5	447A	ALA	3.46	728	207
6	447A	ALA	3.26	738	207
7	502A	VAL	3.55	739	651
8	505A	ALA	3.21	749	669
9	506A	ALA	3.41	751	675

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	432A	ASP	2.55	3.41	171.33	✔	✖	84 [Nam]	755 [N3]
2	443A	GLN	2.05	2.86	158.58	✔	✔	175 [Nam]	730 [Nar]
3	502A	VAL	3.52	3.92	107.11	✖	✖	723 [Npl]	649 [O2]
4	502A	VAL	2.04	2.79	131.97	✖	✖	724 [Npl]	649 [O2]

11. ZINC000001612996

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	450A	HIS	3.98	761	239
2	454A	GLN	3.61	724	285

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	449A	ARG	3.77	4.04	102.21	✔	✔	227 [Ng+]	757 [N3]
2	457A	ASN	2.03	2.84	157.39	✔	✖	305 [Nam]	741 [O2]

12. ZINC000003780340

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	454A	GLN	3.80	721	285
2	454A	GLN	3.86	723	284
3	456A	PRO	3.08	739	302
4	494A	TRP	3.97	725	561

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	451A	ARG	2.01	2.84	163.41	✔	✔	257 [Ng+]	755 [O3]
2	451A	ARG	2.04	2.89	169.96	✔	✔	258 [Ng+]	727 [O2]
3	454A	GLN	2.92	3.55	123.62	✖	✔	753 [O3]	287 [O2]
4	457A	ASN	2.21	3.07	179.56	✔	✖	305 [Nam]	759 [O3]
5	457A	ASN	3.06	3.86	140.45	✖	✔	759 [O3]	311 [O2]
6	494A	TRP	3.36	4.05	139.47	✔	✔	558 [Nar]	730 [O2]

13. ZINC000028827350

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	506A	ALA	3.95	724	675

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	432A	ASP	2.86	3.45	127.17	✔	✖	84 [Nam]	732 [Nam]
2	433A	ALA	2.11	2.96	170.06	✔	✖	93 [Nam]	731 [O2]
3	441A	HIS	2.86	3.16	102.47	✔	✔	156 [Npl]	731 [O2]
4	444A	THR	3.47	3.94	118.73	✔	✔	184 [O3]	719 [O2]
5	503A	ALA	1.95	2.65	127.41	✖	✖	721 [Nam]	657 [O2]
6	505A	ALA	2.62	3.34	141.94	✔	✖	665 [Nam]	721 [Nam]

Table A-2. Interacting residues and amino acids of identified compounds against *GlfT2*.

1. CSID541554

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	309B	TRP	3.81	6133	2919
2	344B	TYR	3.34	6135	3287
3	347B	TRP	3.60	6112	3312
4	369B	LYS	3.94	6131	3529
5	370B	TRP	3.54	6133	3547
6	372B	ASP	3.59	6112	3565
7	373B	ALA	3.15	6134	3574
8	399B	TRP	3.92	6122	3814
9	399B	TRP	3.47	6123	3816

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	370B	TRP	2.11	2.99	142.90	✔	✖	3535 [Nam]	6108 [O2]

2. CSID67239

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	236B	TYR	3.53	6117	2208
2	309B	TRP	3.26	6127	2928
3	309B	TRP	3.52	6128	2927
4	309B	TRP	3.71	6134	2918
5	344B	TYR	3.76	6133	3287
6	348B	TRP	3.35	6121	3335
7	348B	TRP	3.83	6130	3337
8	368B	ILE	3.47	6115	3520
9	369B	LYS	3.47	6136	3529
10	370B	TRP	3.46	6135	3541
11	370B	TRP	3.48	6134	3543
12	373B	ALA	3.56	6133	3574
13	399B	TRP	3.59	6127	3816

3. DB12983

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	317A	TYR	3.29	6132	2994
2	317A	TYR	3.79	6133	2991
3	318A	ASP	3.60	6137	3005
4	399A	TRP	3.95	6118	3815
5	399A	TRP	3.92	6126	3807
6	403A	ASP	3.61	6142	3853
7	405A	ALA	3.55	6144	3871

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	399A	TRP	2.48	3.49	169.06	✔	✔	3811 [Nar]	6110 [N2]
2	401A	ASP	3.11	4.06	155.41	✔	✖	3826 [Nam]	6114 [Nar]

4. DB12424

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	399A	TRP	2.95	6126	3814
2	403A	ASP	3.11	6116	3853
3	404A	ASP	3.38	6139	3862
4	405A	ALA	3.07	6127	3871
5	406A	ILE	3.09	6139	3880

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	369A	LYS	2.21	3.02	134.50	✔	✔	3531 [N3+]	6113 [Nam]
2	405A	ALA	3.06	4.05	164.32	✔	✖	3866 [Nam]	6109 [Nam]
3	409A	GLN	3.11	3.60	111.16	✔	✔	3915 [Nam]	6113 [Nam]
4	409A	GLN	2.12	3.06	154.74	✖	✔	6113 [Nam]	3914 [O2]
5	451A	GLU	2.20	3.07	142.88	✖	✔	6133 [N3]	4333 [O3]

5. DB04016

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	406A	ILE	3.66	6111	3879
2	445A	LYS	3.40	6116	4274
3	445A	LYS	3.09	6117	4273
4	550A	THR	3.17	6148	5282
5	554A	ARG	3.16	6151	5316

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	405A	ALA	1.85	2.63	130.94	✓	×	3866 [Nam]	6118 [O2]
2	451A	GLU	3.29	3.98	132.34	✓	✓	4333 [O3]	6154 [O2]
3	550A	THR	2.42	3.12	127.70	×	×	6157 [O3]	5278 [O2]
4	553A	ALA	2.05	2.99	158.80	×	×	6155 [O3]	5308 [O2]
5	577A	ARG	2.61	3.47	142.06	✓	✓	5528 [Ng+]	6155 [O3]
6	577A	ARG	2.34	3.27	150.96	✓	✓	5525 [Ng+]	6155 [O3]

6. DB08827

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	236A	TYR	3.94	6135	2208
2	347A	TRP	3.13	6135	3312
3	348A	TRP	3.93	6113	3335
4	368A	ILE	3.12	6150	3520
5	397A	MET	3.29	6122	3792

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	256A	ASP	3.10	3.70	119.04	×	✓	6115 [Nam]	2413 [O2]
2	369A	LYS	2.66	3.14	108.96	✓	×	3522 [Nam]	6151 [O2]
3	370A	TRP	2.66	3.19	112.60	✓	×	3535 [Nam]	6151 [O2]
4	396A	HIS	2.93	3.33	103.94	✓	✓	3785 [Npl]	6115 [Nam]

7. DB12154

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	309A	TRP	3.60	6115	2926
2	399A	TRP	3.96	6134	3808
3	403A	ASP	3.35	6146	3853
4	405A	ALA	3.96	6146	3871

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	344A	TYR	1.75	2.66	159.47	✔	✔	3289 [O3]	6121 [Nar]
2	401A	ASP	2.83	3.59	131.68	✔	✖	3826 [Nam]	6138 [Nar]

8. DB15637

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	348A	TRP	3.83	6111	3337
2	369A	LYS	3.74	6140	3529

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	296A	HIS	2.79	3.22	105.87	✔	✔	2798 [Npl]	6115 [O2]
2	344A	TYR	2.21	3.00	139.19	✔	✔	3289 [O3]	6118 [Nar]
3	347A	TRP	1.95	2.87	149.73	✔	✖	3307 [Nam]	6131 [O2]
4	348A	TRP	3.36	3.72	102.81	✔	✖	3323 [Nam]	6133 [Nam]
5	372A	ASP	2.53	3.03	113.30	✔	✔	3568 [O3]	6131 [O2]

9. DB03044

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	318A	ASP	3.76	6144	3005
2	399A	TRP	3.69	6121	3807
3	401A	ASP	3.76	6146	3831
4	403A	ASP	3.71	6121	3853

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	401A	ASP	3.21	3.92	127.40	✓	×	3826 [Nam]	6109 [Nam]
2	403A	ASP	3.19	3.88	126.53	×	×	6110 [Nam]	3852 [O2]
3	403A	ASP	2.96	3.51	114.94	×	×	6109 [Nam]	3852 [O2]

10. DB06589

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	169A	PHE	3.79	6135	1592
2	236A	TYR	3.81	6134	2208
3	348A	TRP	3.83	6115	3335
4	348A	TRP	3.86	6125	3337

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	169A	PHE	3.64	4.10	109.29	✓	×	1587 [Nam]	6138 [O2]
2	171A	ARG	2.88	3.78	146.96	✓	✓	1618 [Ng+]	6138 [O2]
3	171A	ARG	2.69	3.62	151.49	✓	✓	1624 [Ng+]	6138 [O2]
4	236A	TYR	2.10	2.83	127.06	×	✓	6109 [Npl]	2210 [O3]
5	256A	ASP	2.42	3.44	171.86	×	✓	6139 [N3]	2412 [O3]
6	370A	TRP	1.95	2.91	155.83	✓	×	3535 [Nam]	6119 [Nar]

11. DB12228

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	167A	PRO	3.38	6143	1575
2	169A	PHE	3.47	6144	1592
3	236A	TYR	3.41	6133	2208
4	348A	TRP	3.37	6125	3337
5	369A	LYS	3.62	6113	3529

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	296A	HIS	2.10	2.99	145.21	✔	✔	2798 [Npl]	6121 [Nar]
2	300A	GLU	2.21	3.00	133.71	✖	✔	6120 [Nam]	2832 [O2]
3	344A	TYR	3.07	3.95	155.12	✔	✔	3289 [O3]	6120 [Nam]
4	369A	LYS	3.34	4.09	131.56	✔	✖	3522 [Nam]	6108 [O2]

12. DB11691

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	309A	TRP	3.59	6131	2928
2	397A	MET	3.70	6153	3792
3	399A	TRP	3.44	6125	3814
4	399A	TRP	3.67	6116	3807
5	399A	TRP	3.92	6139	3808
6	405A	ALA	3.48	6132	3871

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	296A	HIS	2.68	3.54	141.99	✔	✔	2798 [Npl]	6148 [O3]
2	369A	LYS	2.21	3.20	162.79	✔	✔	3531 [N3+]	6146 [O2]
3	405A	ALA	2.99	3.72	129.67	✔	✖	3866 [Nam]	6135 [Nar]
4	413A	HIS	2.29	3.28	163.21	✔	✔	3961 [Npl]	6146 [O2]

13. DB13676

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	348A	TRP	3.10	6134	3337
2	399A	TRP	3.47	6138	3816
3	399A	TRP	3.15	6142	3814
4	399A	TRP	3.57	6123	3807
5	403A	ASP	3.34	6117	3853
6	405A	ALA	3.89	6120	3871

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	369A	LYS	1.75	2.74	161.25	✔	✔	3531 [N3+]	6110 [O2]
2	413A	HIS	2.06	2.82	130.05	✔	✔	3961 [Npl]	6110 [O2]

14. DB01988

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	399A	TRP	3.31	6130	3814
2	399A	TRP	3.96	6127	3807
3	403A	ASP	3.19	6127	3853
4	554A	ARG	3.28	6140	5316

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	401A	ASP	3.05	3.96	149.97	✔	✖	3826 [Nam]	6131 [Nar]
2	405A	ALA	1.93	2.87	151.41	✔	✖	3866 [Nam]	6111 [Nar]
3	553A	ALA	3.01	3.45	107.47	✖	✖	6109 [N3]	5308 [O2]

15. DB08815

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	309A	TRP	3.34	6122	2921
2	344A	TYR	3.32	6123	3287
3	405A	ALA	3.46	6139	3871

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	369A	LYS	2.51	3.39	143.64	✔	✔	3531 [N3+]	6116 [O2]
2	413A	HIS	1.94	2.94	166.26	✔	✔	3961 [Npl]	6116 [O2]

16. DB14773

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	344A	TYR	2.28	2.90	118.28	×	✔	6134 [Nam]	3289 [O3]
2	344A	TYR	2.32	3.25	164.24	✔	✔	3289 [O3]	6135 [Nar]
3	370A	TRP	3.05	3.58	113.01	✔	×	3535 [Nam]	6133 [O2]
4	373A	ALA	2.79	3.49	126.48	✔	×	3569 [Nam]	6133 [O2]
5	403A	ASP	2.98	3.49	115.62	✔	✔	3856 [O3]	6108 [O3]
6	403A	ASP	2.12	2.75	118.62	×	×	6118 [Npl]	3852 [O2]
7	405A	ALA	2.72	3.52	135.17	✔	×	3866 [Nam]	6119 [N2]

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	309A	TRP	3.97	6141	2919
2	309A	TRP	3.87	6114	2926
3	369A	LYS	3.98	6138	3529
4	370A	TRP	3.72	6141	3541
5	399A	TRP	3.43	6139	3816
6	399A	TRP	3.82	6110	3807
7	404A	ASP	3.86	6123	3862
8	405A	ALA	3.90	6124	3871

17. DB14703

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	236A	TYR	3.14	6127	2208
2	347A	TRP	3.88	6125	3312
3	348A	TRP	3.94	6120	3335
4	348A	TRP	3.63	6121	3337
5	399A	TRP	2.99	6142	3816

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	344A	TYR	3.27	3.70	110.03	✓	✓	3289 [O3]	6147 [O3]
2	344A	TYR	3.17	3.70	116.12	×	✓	6147 [O3]	3289 [O3]
3	369A	LYS	3.30	3.88	118.18	✓	✓	3531 [N3+]	6130 [O3]
4	370A	TRP	1.71	2.68	157.69	✓	×	3535 [Nam]	6148 [O3]
5	372A	ASP	2.32	2.78	107.18	×	✓	6149	3568 [O3]

18. DB04868

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	169A	PHE	3.76	6125	1594
2	399A	TRP	3.30	6137	3814
3	403A	ASP	3.60	6144	3853

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	171A	ARG	3.00	4.02	174.93	✓	✓	1624 [Ng+]	6116 [Nar]
2	171A	ARG	2.23	3.20	159.49	✓	✓	1618 [Ng+]	6117 [Nar]
3	258A	ASP	2.76	3.71	156.95	×	✓	6115 [Npl]	2430 [O3]
4	369A	LYS	2.97	3.63	123.90	✓	✓	3531 [N3+]	6140 [Nar]

19. DB01092

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	236A	TYR	3.81	6115	2208
2	368A	ILE	3.40	6119	3520
3	399A	TRP	3.77	6147	3816

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	169A	PHE	2.00	2.64	118.30	✓	×	1587 [Nam]	6127 [O2]
2	236A	TYR	2.19	3.10	154.97	×	✓	6136 [O3]	2210 [O3]
3	344A	TYR	1.91	2.80	154.18	✓	✓	3289 [O3]	6152 [O3]
4	344A	TYR	2.09	2.80	128.06	×	✓	6152 [O3]	3289 [O3]
5	369A	LYS	2.22	3.15	150.42	✓	×	3522 [Nam]	6155 [O3]
6	370A	TRP	2.27	3.13	140.86	✓	×	3535 [Nam]	6148 [O3]
7	371A	ASP	3.30	4.05	134.59	×	✓	6138 [O3]	3559 [O3]
8	371A	ASP	2.05	2.73	125.20	×	✓	6155 [O3]	3558 [O2]
9	371A	ASP	2.75	3.38	122.90	×	✓	6134 [O3]	3558 [O2]
10	372A	ASP	3.17	3.95	134.49	✓	×	3560	6138 [O3]

20. DB01337

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	309A	TRP	3.74	6119	2927
2	309A	TRP	3.02	6113	2928
3	309A	TRP	3.24	6123	2926
4	311A	ALA	3.95	6132	2943
5	317A	TYR	3.29	6108	2996
6	399A	TRP	3.63	6139	3807
7	405A	ALA	3.54	6138	3871

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	309A	TRP	2.79	3.34	114.17	✓	✓	2922 [Nar]	6146 [O2]
2	317A	TYR	2.14	3.07	151.21	✓	×	2986 [Nam]	6142 [O2]

21. DB00872

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	167A	PRO	3.33	6140	1575
2	169A	PHE	3.41	6139	1592
3	309A	TRP	3.65	6129	2919
4	309A	TRP	3.99	6127	2921
5	348A	TRP	3.56	6130	3338
6	367A	PHE	3.53	6145	3511
7	368A	ILE	3.46	6112	3520
8	369A	LYS	3.65	6126	3527
9	399A	TRP	3.87	6130	3816

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	232A	GLY	2.31	2.76	104.86	✔	✖	2175 [Nam]	6132 [O2]
2	369A	LYS	2.91	3.51	118.58	✔	✖	3522 [Nam]	6114 [O2]
3	370A	TRP	3.11	3.65	113.88	✔	✖	3535 [Nam]	6114 [O2]

22. ZINC000043203371

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	399A	TRP	3.72	6149	3814
2	399A	TRP	3.57	6146	3810
3	399A	TRP	3.63	6145	3807
4	403A	ASP	3.56	6152	3853

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	309A	TRP	2.51	3.38	149.22	✖	✖	6123 [Nam]	2917 [O2]
2	311A	ALA	1.77	2.78	168.63	✔	✖	2938 [Nam]	6125 [Nar]
3	403A	ASP	1.81	2.74	151.21	✖	✖	6140 [N3]	3852 [O2]

23. ZINC000063933734

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	169A	PHE	3.49	6146	1592
2	169A	PHE	3.91	6139	1593
3	169A	PHE	3.68	6151	1596
4	368A	ILE	3.12	6116	3520
5	408A	TRP	3.60	6134	3905
6	408A	TRP	3.38	6149	3903
7	480A	LEU	3.24	6151	4598

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	200A	GLN	2.37	3.04	122.22	✔	✔	1873 [Nam]	6142 [Nar]
2	229A	ASN	2.96	3.83	143.20	✔	✔	2158 [Nam]	6136 [Nar]
3	370A	TRP	2.07	3.08	176.35	✔	✖	3535 [Nam]	6131 [O2]

24. ZINC000095092808

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	309A	TRP	3.21	6109	2926
2	369A	LYS	3.53	6112	3529
3	399A	TRP	3.69	6136	3807

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	232A	GLY	2.58	3.10	111.33	✔	✖	2175 [Nam]	6123 [Nar]
2	233A	SER	3.15	3.64	111.41	✔	✖	2180 [Nam]	6124 [Nar]
3	369A	LYS	1.99	2.89	145.45	✔	✖	3522 [Nam]	6125 [Nar]
4	369A	LYS	2.75	3.77	176.39	✔	✔	3531 [N3+]	6126 [N2]
5	371A	ASP	1.87	2.72	142.11	✖	✔	6125 [Nar]	3558 [O2]
6	413A	HIS	2.60	3.58	160.75	✔	✔	3961 [Npl]	6127 [N2]

25. ZINC000027990463

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	309A	TRP	3.98	6119	2926
2	309A	TRP	3.42	6141	2928
3	317A	TYR	3.68	6143	2996
4	344A	TYR	3.23	6122	3287
5	399A	TRP	3.08	6129	3816
6	399A	TRP	3.40	6142	3815
7	399A	TRP	3.55	6138	3808
8	399A	TRP	3.23	6136	3807
	403A	ASP	3.64	6136	3853

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	309A	TRP	3.60	3.99	105.40	✔	✔	2922 [Nar]	6115 [O2]
2	369A	LYS	2.86	3.58	127.97	✔	✔	3531 [N3+]	6115 [O2]
3	413A	HIS	1.91	2.76	138.75	✔	✔	3961 [Npl]	6115 [O2]

26. ZINC000001612996

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	169A	PHE	3.94	6136	1592
2	309A	TRP	3.52	6149	2921
3	344A	TYR	3.60	6150	3287
4	348A	TRP	3.61	6144	3337
5	399A	TRP	3.83	6142	3814

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	200A	GLN	2.61	3.39	132.92	✔	✔	1873 [Nam]	6127 [O2]
2	396A	HIS	2.66	3.41	130.43	✔	✔	3781 [Npl]	6139 [O2]

27. ZINC000253633622

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	399A	TRP	3.17	6148	3807
2	403A	ASP	3.37	6147	3853
3	404A	ASP	3.86	6117	3862
4	405A	ALA	3.83	6146	3871
5	406A	ILE	3.19	6128	3879
6	406A	ILE	3.57	6117	3880
7	550A	THR	3.58	6119	5282
	566A	VAL	2.89	6119	5432

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	401A	ASP	2.79	3.15	100.83	✔	✖	3826 [Nam]	6135 [O3]
2	403A	ASP	2.58	3.48	146.57	✔	✖	3848 [Nam]	6135 [O3]
3	403A	ASP	2.33	2.94	120.22	✖	✖	6135 [O3]	3852 [O2]
4	403A	ASP	2.01	2.89	144.26	✖	✖	6108 [N3]	3852 [O2]
5	405A	ALA	2.05	3.05	164.47	✔	✖	3866 [Nam]	6144 [Nar]

28. ZINC000043204146

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	309A	TRP	3.93	6138	2926
2	369A	LYS	3.44	6142	3529
3	399A	TRP	3.45	6124	3814
4	399A	TRP	3.72	6151	3808

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	369A	LYS	2.12	3.12	165.01	✔	✔	3531 [N3+]	6109 [O2]
2	370A	TRP	2.16	3.17	170.85	✔	✖	3535 [Nam]	6140 [O3]
3	413A	HIS	3.05	4.00	155.91	✔	✔	3961 [Npl]	6135 [Npl]

29. ZINC000100378061

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	236A	TYR	2.95	6150	2208
2	347A	TRP	3.74	6150	3319
3	347A	TRP	3.62	6151	3312
4	368A	ILE	3.32	6118	3520
5	397A	MET	3.15	6131	3792
6	399A	TRP	3.38	6142	3816

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	171A	ARG	2.56	3.13	114.95	✓	✓	1618 [Ng+]	6122 [O2]
2	171A	ARG	2.20	2.82	117.45	✓	✓	1624 [Ng+]	6122 [O2]
3	236A	TYR	2.70	3.57	150.03	×	✓	6145 [O3]	2210 [O3]
4	403A	ASP	3.15	3.45	101.11	✓	✓	3856 [O3]	6137 [Nar]

30. ZINC000003978005

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	399A	TRP	3.24	6109	3807
2	403A	ASP	3.18	6109	3853
3	404A	ASP	3.90	6130	3862
4	406A	ILE	3.29	6142	3879
5	550A	THR	3.95	6140	5282
6	569A	ALA	3.61	6151	5457

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	401A	ASP	3.15	3.66	112.43	✓	×	3826 [Nam]	6122 [O2]
2	403A	ASP	2.14	2.68	113.49	×	×	6125 [Nam]	3852 [O2]
3	403A	ASP	3.06	3.90	140.59	✓	×	3848 [Nam]	6122 [O2]
4	405A	ALA	3.31	3.95	122.17	✓	×	3866 [Nam]	6128 [N3]
5	553A	ALA	2.01	2.73	129.33	×	×	6137 [Npl]	5308 [O2]

Table A-3. Interacting residues and amino acids of identified compounds against *MurB.*

1. CSID1438694

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	71A	ASN	3.50	3236	588
2	72A	LEU	2.99	3222	601
3	127A	ILE	3.40	3232	1078
4	133A	ALA	3.38	3226	1116
5	136A	VAL	3.20	3243	1140
6	136A	VAL	3.43	3251	1139
7	139A	VAL	3.23	3233	1171
8	188A	VAL	3.36	3247	1652
9	188A	VAL	3.03	3245	1651

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	68A	GLY	2.15	3.16	168.78	✔	✖	565 [Nam]	3228 [O2]
2	130A	SER	2.09	3.05	156.67	✔	✖	1092 [Nam]	3228 [O2]

2. CSID2166135

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	26A	THR	3.75	3231	185
2	127A	ILE	3.79	3239	1078
3	128A	PRO	3.82	3232	1085
4	141A	ALA	2.95	3246	1182
5	245A	LEU	2.90	3231	2172

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	70A	SER	2.08	2.90	145.81	✖	✖	3234 [Npl]	579 [O2]
2	238A	ARG	3.39	4.05	124.18	✔	✔	2103 [Ng+]	3224 [Npl]
3	257A	SER	2.40	3.12	127.26	✔	✖	2275 [Nam]	3253 [O2]
4	361A	GLU	2.45	3.24	142.43	✔	✔	3156 [O3]	3220 [O2]

3. CSID1655442

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	175A	TYR	3.06	3242	1527
2	176A	ARG	3.87	3241	1538
3	262A	PRO	3.62	3251	2331
4	364A	LEU	3.16	3234	3177

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	177A	THR	3.70	3.99	101.39	✔	✔	1556 [O3]	3223 [O2]
2	181A	LYS	2.18	2.84	120.27	✔	✔	1593 [N3+]	3223 [O2]
3	359A	LYS	2.18	3.09	148.38	✔	✖	3134 [Nam]	3248 [O2]

4. CSID2154128

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	141A	ALA	3.22	3233	1182
2	142A	TYR	3.70	3235	1193
3	145A	GLU	3.28	3226	1214
4	175A	TYR	3.73	3226	1528
5	241A	LYS	3.29	3237	2136
6	296A	ALA	3.64	3253	2579

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	140A	GLY	2.78	3.71	174.52	✖	✖	3230 [Npl]	1176 [O2]
2	142A	TYR	3.19	3.89	127.66	✔	✖	1183 [Nam]	3230 [Npl]
3	175A	TYR	3.08	3.62	117.32	✔	✔	1531 [O3]	3228 [O2]
4	176A	ARG	2.62	3.55	151.03	✔	✔	1547 [Ng+]	3230 [Npl]
5	210A	TYR	2.33	3.18	151.05	✖	✔	3240 [Npl]	1854 [O3]
6	261A	ASN	2.16	3.05	144.77	✔	✔	2324 [Nam]	3228 [O2]
7	294A	LYS	3.15	3.54	104.26	✔	✔	2561 [N3+]	3228 [O2]

5. CSID2165834

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	128A	PRO	3.08	3231	1085
2	137A	GLN	3.51	3240	1146
3	139A	VAL	3.04	3240	1171
4	141A	ALA	3.90	3249	1182
5	361A	GLU	3.61	3239	3153

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	140A	GLY	2.36	3.33	158.22	✔	✖	1172 [Nam]	3220 [O2]
2	238A	ARG	1.59	2.57	161.42	✔	✔	2100 [Ng+]	3252 [O2]
3	238A	ARG	3.06	4.01	155.39	✔	✔	2103 [Ng+]	3224 [Npl]

6. CSID2156566

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	127A	ILE	3.88	3225	1078
2	128A	PRO	3.64	3233	1085
3	141A	ALA	3.11	3243	1182
4	245A	LEU	3.52	3234	2172

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	70A	SER	2.03	2.81	140.14	✖	✖	3230 [Npl]	579 [O2]
2	238A	ARG	3.42	4.06	122.66	✔	✔	2103 [Ng+]	3238 [Npl]
3	257A	SER	2.56	3.24	123.65	✔	✖	2275 [Nam]	3249 [O2]
4	361A	GLU	2.49	3.31	145.91	✔	✔	3156 [O3]	3228 [O2]

7. CSID2140363

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	67A	ALA	3.40	3239	564
2	71A	ASN	3.41	3237	588
3	72A	LEU	3.87	3241	601
4	133A	ALA	3.35	3239	1116
5	136A	VAL	3.67	3250	1140
6	136A	VAL	3.21	3230	1139
7	137A	GLN	3.47	3232	1147
8	182A	HIS	3.99	3228	1602
9	188A	VAL	2.97	3244	1651
10	188A	VAL	3.25	3227	1652
11	363A	VAL	3.06	3232	3171

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	67A	ALA	3.41	3.91	112.42	✓	×	559 [Nam]	3220 [O2]
2	71A	ASN	2.78	3.75	159.58	✓	✓	591 [Nam]	3234 [Npl]
3	192A	VAL	2.33	3.29	157.32	✓	×	1677 [Nam]	3249 [O3]

8. CSID2156621

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	175A	TYR	3.10	3232	1527
2	175A	TYR	3.78	3231	1524
3	176A	ARG	3.83	3250	1538
4	260A	THR	3.56	3251	2315
5	263A	VAL	3.28	3232	2341
6	263A	VAL	3.56	3229	2339
7	356A	ILE	3.35	3242	3114
8	359A	LYS	3.66	3248	3139
9	360A	PRO	3.64	3256	3145

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	181A	LYS	2.27	2.75	107.19	✓	✓	1593 [N3+]	3252 [O3]
2	261A	ASN	2.73	3.21	112.67	×	×	3234 [Npl]	2320 [O2]
3	263A	VAL	2.90	3.83	152.05	✓	×	2334 [Nam]	3226 [Npl]
4	357A	THR	2.35	3.30	154.99	✓	×	3116 [Nam]	3244 [O3]
5	359A	LYS	2.31	3.25	153.84	✓	×	3134 [Nam]	3220 [O2]

9. CSID3866834

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	141A	ALA	3.37	3221	1182
2	296A	ALA	3.53	3229	2579
3	326A	LEU	3.20	3231	2846

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	142A	TYR	1.92	2.86	152.04	✔	✖	1183 [Nam]	3236 [O2]
2	210A	TYR	2.04	2.86	145.32	✖	✔	3225 [Npl]	1854 [O3]
3	257A	SER	2.97	3.91	174.85	✔	✔	2281 [O3]	3233 [Npl]
4	257A	SER	3.36	3.91	120.23	✖	✔	3233 [Npl]	2281 [O3]

10. CSID2158441

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	145A	GLU	3.26	3223	1214
2	175A	TYR	3.86	3252	1524
3	210A	TYR	3.68	3234	1851
4	287A	TYR	3.45	3233	2509

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	142A	TYR	2.16	2.88	125.46	✔	✖	1183 [Nam]	3247 [O2]
2	143A	GLY	2.99	3.95	158.89	✔	✖	1197 [Nam]	3247 [O2]
3	294A	LYS	2.60	3.50	147.03	✔	✔	2561 [N3+]	3256 [O3]

11. CSID2156999

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	360A	PRO	3.63	3246	3145
2	363A	VAL	3.48	3244	3170
3	364A	LEU	3.66	3248	3177

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	183A	ALA	2.96	3.78	146.85	×	×	3224 [Npl]	1614 [O2]
2	366A	GLY	2.13	2.76	118.40	✓	×	3190 [Nam]	3220 [O2]
3	367A	CYS	2.37	3.35	162.06	✓	×	3195 [Nam]	3220 [O2]

12. DB15688

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	127A	ILE	3.36	3238	1079
2	127A	ILE	3.52	3240	1078
3	139A	VAL	3.75	3240	1170
4	141A	ALA	3.46	3234	1182
5	175A	TYR	3.85	3254	1528
6	210A	TYR	3.05	3253	1851

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	176A	ARG	2.20	3.11	147.29	✓	✓	1547 [Ng+]	3232 [Nam]
2	210A	TYR	2.30	2.99	124.47	×	✓	3223 [Nam]	1854 [O3]
3	257A	SER	2.03	2.78	133.60	✓	✓	2281 [O3]	3231 [O2]
4	261A	ASN	2.78	3.16	102.77	✓	✓	2324 [Nam]	3244 [N3]
5	302A	GLU	3.00	3.94	154.08	×	✓	3259 [Npl]	2632 [O2]
6	325A	ALA	2.18	3.13	154.10	✓	×	2833 [Nam]	3260 [N2]
7	361A	GLU	2.27	3.14	142.43	×	✓	3232 [Nam]	3156 [O3]

13. DB12983

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	145A	GLU	3.85	3259	1214
2	261A	ASN	3.97	3247	2321
3	287A	TYR	3.36	3245	2509
4	288A	PRO	3.42	3252	2515
5	296A	ALA	3.68	3251	2579

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	210A	TYR	3.56	4.05	112.54	✗	✓	3221 [N2]	1854 [O3]
2	261A	ASN	2.99	3.42	106.36	✓	✓	2324 [Nam]	3224 [N2]
3	294A	LYS	2.19	3.21	172.37	✓	✓	2561 [N3+]	3227 [N2]

14. DB14773

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	175A	TYR	3.12	3238	1527
2	263A	VAL	3.87	3238	2341
3	359A	LYS	3.89	3221	3139
4	360A	PRO	3.59	3250	3145
5	364A	LEU	3.62	3253	3180

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	181A	LYS	3.29	3.84	115.39	✓	✓	1593 [N3+]	3220 [O3]
2	185A	GLY	2.91	3.61	126.87	✓	✗	1625 [Nam]	3247 [Nar]
3	260A	THR	2.97	3.87	159.30	✓	✓	2313 [O3]	3231 [N2]
4	364A	LEU	2.28	3.23	155.06	✓	✗	3172 [Nam]	3247 [Nar]
5	364A	LEU	2.12	2.83	124.52	✗	✗	3246 [Nam]	3176 [O2]

15. DB06229

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	67A	ALA	3.03	3233	564
2	71A	ASN	3.15	3223	588
3	72A	LEU	3.70	3234	600
4	127A	ILE	3.91	3245	1078
5	128A	PRO	3.65	3246	1085
6	133A	ALA	3.86	3230	1116
7	243A	MET	3.72	3250	2152

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	68A	GLY	3.08	3.97	145.99	✔	✖	565 [Nam]	3228 [Nar]
2	130A	SER	2.13	3.10	158.63	✔	✖	1092 [Nam]	3228 [Nar]
3	238A	ARG	2.90	3.68	133.44	✔	✔	2103 [Ng+]	3240 [Nar]

16. DB12424

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	183A	ALA	3.79	3259	1615
2	184A	ASP	3.43	3236	1621
3	363A	VAL	3.37	3250	3171

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	364A	LEU	2.32	3.16	139.34	✔	✖	3172 [Nam]	3244 [O2]
2	366A	GLY	2.65	3.56	148.87	✔	✖	3190 [Nam]	3224 [O2]

17. DB15396

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	141A	ALA	3.27	3225	1182
2	145A	GLU	3.25	3240	1214
3	175A	TYR	3.80	3240	1528

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	143A	GLY	2.14	3.04	146.05	✔	✖	1197 [Nam]	3234 [Nar]
2	238A	ARG	1.95	2.86	147.40	✔	✔	2100 [Ng+]	3220 [O2]
3	238A	ARG	2.16	3.04	143.01	✔	✔	2103 [Ng+]	3220 [O2]
4	361A	GLU	3.32	3.98	123.89	✖	✔	3221 [Nam]	3156 [O3]

18. DB15401

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	127A	ILE	3.92	3255	1078
2	175A	TYR	3.91	3239	1528
3	210A	TYR	3.59	3227	1851

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	143A	GLY	2.11	3.00	143.50	✔	✖	1197 [Nam]	3237 [O2]
2	176A	ARG	3.39	4.01	120.79	✔	✔	1544 [Ng+]	3222 [Nar]
3	176A	ARG	2.04	2.98	151.19	✔	✔	1547 [Ng+]	3222 [Nar]
4	238A	ARG	1.91	2.51	114.19	✔	✔	2100 [Ng+]	3249 [O2]
5	238A	ARG	3.41	3.80	104.77	✔	✔	2103 [Ng+]	3249 [O2]
6	257A	SER	2.13	3.06	165.57	✔	✔	2281 [O3]	3241 [O2]
7	257A	SER	3.10	4.02	150.90	✖	✔	3250 [Nam]	2281 [O3]
8	257A	SER	2.87	3.74	143.62	✔	✖	2275 [Nam]	3251 [Nar]

19. DB03461

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	263A	VAL	3.94	3238	2339

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	175A	TYR	2.01	2.99	160.38	✓	×	1519 [Nam]	3258 [Nar]
2	260A	THR	1.84	2.74	156.77	✓	✓	2313 [O3]	3228 [O3]
3	263A	VAL	2.62	3.42	135.43	✓	×	2334 [Nam]	3230 [O3]
4	291A	ASP	3.08	3.98	153.60	×	✓	3274 [O3]	2537 [O3]
5	291A	ASP	3.35	3.70	103.79	×	✓	3276 [O3]	2538 [O2]
6	357A	THR	2.91	3.84	159.14	×	×	3246 [O3]	3120 [O2]
7	357A	THR	2.22	2.89	125.43	×	×	3248 [O3]	3120 [O2]
8	359A	LYS	2.09	3.10	170.27	✓	×	3134 [Nam]	3246 [O3]
9	360A	PRO	3.34	3.64	100.08	×	×	3227	3143 [O2]

20. DB11852

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	184A	ASP	3.79	3253	1621
2	186A	LEU	3.97	3254	1635
3	359A	LYS	3.62	3230	3139
4	360A	PRO	3.10	3224	3145
5	364A	LEU	3.93	3248	3177

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	181A	LYS	2.81	3.34	113.01	✓	✓	1593 [N3+]	3221 [Nar]
2	185A	GLY	2.28	3.26	161.54	✓	×	1625 [Nam]	3242 [Nar]

21. DB08901

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	136A	VAL	3.80	3239	1139
2	181A	LYS	3.48	3232	1591
3	188A	VAL	3.40	3240	1652
4	360A	PRO	3.26	3226	3145
5	363A	VAL	3.43	3232	3171
6	363A	VAL	3.76	3230	3170

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	181A	LYS	2.48	3.13	121.29	✔	✔	1593 [N3+]	3221 [Nam]
2	182A	HIS	2.15	2.97	135.37	✔	✖	1597 [Nam]	3235 [Nar]
3	263A	VAL	2.69	3.67	160.54	✔	✖	2334 [Nam]	3254 [N3]

22. ZINC003975327

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	175A	TYR	3.14	3220	1524
2	175A	TYR	3.44	3228	1529
3	263A	VAL	3.26	3220	2341

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	175A	TYR	3.23	3.73	111.53	✔	✖	1519 [Nam]	3261 [Nar]
2	177A	THR	3.00	3.30	100.47	✔	✔	1556 [O3]	3241 [Nar]

23. ZINC254071113

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	210A	TYR	3.20	3248	1849
2	283A	PRO	3.34	3274	2473
3	287A	TYR	3.18	3235	2509

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	261A	ASN	2.25	3.08	138.23	✓	✓	2324 [Nam]	3253 [O.co2]
2	284A	VAL	1.87	2.77	154.36	×	×	3270 [Npl]	2480 [O2]
3	286A	HIS	2.80	3.43	120.61	✓	✓	2498 [Npl]	3270 [Npl]
4	298A	GLY	2.48	3.43	154.89	✓	×	2586 [Nam]	3254 [O.co2]

24. ZINC084726167

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	210A	TYR	3.69	3225	1851
2	287A	TYR	3.46	3223	2509
3	296A	ALA	4.00	3228	2579

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	175A	TYR	2.24	3.17	164.73	✓	✓	1531 [O3]	3244 [O3]
2	176A	ARG	3.49	3.84	102.52	✓	✓	1544 [Ng+]	3244 [O3]
3	176A	ARG	2.53	3.01	108.70	✓	✓	1547 [Ng+]	3244 [O3]
4	257A	SER	2.75	3.69	170.47	✓	✓	2281 [O3]	3247 [N3]
5	261A	ASN	1.99	2.97	162.38	✓	✓	2324 [Nam]	3252 [Nam]
6	324A	HIS	3.18	3.70	113.20	✓	✓	2831 [Npl]	3251 [O2]

25. ZINC008215434

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	210A	TYR	3.28	3269	1852
2	212A	GLU	3.64	3269	1866

Hydrogen Bonds ——

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	143A	GLY	2.83	3.29	107.46	✓	×	1197 [Nam]	3249 [O3]
2	144A	ALA	2.14	3.05	155.50	×	×	3249 [O3]	1206 [O2]
3	144A	ALA	2.32	3.26	165.68	×	×	3247 [O3]	1206 [O2]
4	176A	ARG	2.14	3.16	170.79	✓	✓	1544 [Ng+]	3247 [O3]
5	176A	ARG	2.99	3.80	136.53	✓	✓	1547 [Ng+]	3247 [O3]
6	210A	TYR	2.11	2.76	123.22	×	✓	3259 [O3]	1854 [O3]
7	257A	SER	3.16	3.99	146.15	✓	✓	2281 [O3]	3220 [O3]
8	261A	ASN	1.80	2.79	161.20	✓	✓	2324 [Nam]	3223 [O2]
9	294A	LYS	3.60	4.00	105.55	✓	✓	2561 [N3+]	3231 [O3]

26. ZINC095539256

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	175A	TYR	3.00	3259	1528
2	176A	ARG	3.54	3240	1538
3	210A	TYR	3.67	3265	1851
4	260A	THR	3.37	3240	2315
5	290A	PRO	3.68	3280	2527

Hydrogen Bonds ——

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	143A	GLY	2.83	3.29	107.46	✓	×	1197 [Nam]	3249 [O3]
2	144A	ALA	2.14	3.05	155.50	×	×	3249 [O3]	1206 [O2]
3	144A	ALA	2.32	3.26	165.68	×	×	3247 [O3]	1206 [O2]
4	176A	ARG	2.14	3.16	170.79	✓	✓	1544 [Ng+]	3247 [O3]
5	176A	ARG	2.99	3.80	136.53	✓	✓	1547 [Ng+]	3247 [O3]
6	210A	TYR	2.11	2.76	123.22	×	✓	3259 [O3]	1854 [O3]
7	257A	SER	3.16	3.99	146.15	✓	✓	2281 [O3]	3220 [O3]
8	261A	ASN	1.80	2.79	161.20	✓	✓	2324 [Nam]	3223 [O2]
9	294A	LYS	3.60	4.00	105.55	✓	✓	2561 [N3+]	3231 [O3]

27. ZINC003934128

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	172A	ARG	2.98	3259	1491
2	173A	PHE	3.99	3231	1507
3	173A	PHE	3.27	3263	1510
4	290A	PRO	3.49	3271	2528

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	145A	GLU	2.03	2.55	111.12	×	✓	3276 [O3]	1217 [O3]
2	147A	SER	2.10	2.83	130.81	×	×	3268 [O3]	1230 [O2]
3	170A	ASP	3.27	4.00	134.62	×	×	3260 [O3]	1471 [O2]
4	172A	ARG	2.98	3.65	124.19	✓	✓	1493 [Ng+]	3260 [O3]
5	173A	PHE	2.77	3.25	111.06	×	×	3240 [Npl]	1506 [O2]
6	173A	PHE	2.64	3.11	110.42	×	×	3243 [Npl]	1506 [O2]
7	291A	ASP	2.27	3.16	151.79	×	✓	3252 [O3]	2538 [O2]

28. ZINC004215770

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	210A	TYR	3.74	3253	1849
2	287A	TYR	3.70	3227	2509
3	288A	PRO	3.92	3255	2515

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	143A	GLY	2.73	3.34	118.55	✓	×	1197 [Nam]	3240 [N3]
2	176A	ARG	2.94	3.70	131.45	✓	✓	1544 [Ng+]	3245 [O3]
3	176A	ARG	2.19	3.15	156.13	✓	✓	1547 [Ng+]	3245 [O3]
4	210A	TYR	2.84	3.54	130.62	×	✓	3228 [O3]	1854 [O3]
5	261A	ASN	2.03	2.77	126.92	✓	✓	2324 [Nam]	3230 [O3]

29. ZINC003780340

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	210A	TYR	3.63	3220	1849
2	287A	TYR	3.12	3224	2509
3	296A	ALA	3.65	3249	2579

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	140A	GLY	2.74	3.46	133.60	×	×	3242 [O3]	1176 [O2]
2	176A	ARG	2.49	3.33	139.48	✓	✓	1544 [Ng+]	3240 [O2]
3	176A	ARG	2.13	3.09	155.80	✓	✓	1547 [Ng+]	3240 [O2]
4	261A	ASN	1.90	2.88	160.02	✓	✓	2324 [Nam]	3246 [O3]
5	286A	HIS	3.42	4.10	131.41	×	×	3254 [O3]	2495 [O2]

30. ZINC0001893112

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	67A	ALA	3.18	3235	564
2	71A	ASN	3.62	3225	588
3	72A	LEU	3.01	3230	601
4	127A	ILE	3.92	3244	1077
5	133A	ALA	3.02	3224	1116
6	141A	ALA	3.54	3250	1182

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	69A	GLY	2.44	2.94	109.59	✓	×	570 [Nam]	3234 [O3]
2	70A	SER	1.94	2.72	130.54	✓	×	575 [Nam]	3236 [Nox]
3	71A	ASN	2.30	3.32	172.93	✓	×	583 [Nam]	3234 [O3]
4	176A	ARG	2.03	2.95	148.44	✓	✓	1547 [Ng+]	3252 [O3]
5	238A	ARG	2.01	2.98	158.56	✓	✓	2100 [Ng+]	3247 [O3]
6	238A	ARG	2.68	3.49	136.80	✓	✓	2103 [Ng+]	3247 [O3]
7	257A	SER	3.46	4.08	125.65	✓	✓	2281 [O3]	3240 [O2]

31. ZINC003978083

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	145A	GLU	3.73	3234	1214
2	175A	TYR	3.36	3236	1528
3	210A	TYR	3.55	3246	1851
4	210A	TYR	3.28	3258	1849
5	287A	TYR	3.66	3226	2509
6	296A	ALA	3.29	3243	2579

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	143A	GLY	3.44	3.89	109.28	✔	✖	1197 [Nam]	3231 [N3]
2	175A	TYR	2.00	2.91	161.37	✔	✔	1531 [O3]	3263 [O3]
3	211A	GLY	2.11	2.76	118.93	✔	✖	1856 [Nam]	3262 [O3]
4	261A	ASN	2.91	3.92	171.04	✔	✔	2324 [Nam]	3242 [O3]

32. ZINC0011616153

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	360A	PRO	3.36	3224	3145
2	365A	ILE	3.94	3241	3189

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	181A	LYS	2.47	2.92	105.79	✔	✔	1593 [N3+]	3256 [O3]
2	186A	LEU	3.11	3.75	121.97	✔	✖	1630 [Nam]	3240 [O3]
3	364A	LEU	1.90	2.69	131.34	✔	✖	3172 [Nam]	3228 [O2]
4	364A	LEU	3.16	3.79	123.74	✖	✖	3233 [Nam]	3176 [O2]
5	366A	GLY	2.66	3.50	140.33	✔	✖	3190 [Nam]	3240 [O3]

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Original article

Screening and molecular dynamics simulation of compounds inhibiting MurB enzyme of drug-resistant *Mycobacterium tuberculosis*: An *in-silico* approach

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ABSTRACT

Mycobacterium tuberculosis (MTB) is becoming more and more resistant to drugs and it is a common problem, making current antimicrobials ineffective and highlighting the need for new TB drugs. One of the promising targets for treating MTB is MurB enzymes. This study aimed to identify potential inhibitors of MurB enzymes in *M. tuberculosis*, as drug resistance among MTB is a significant problem. Attempts are being made to conduct a virtual screening of 30,417 compounds, and thirty-two compounds were chosen for further analysis based on their binding conformations. The selected compounds were assessed for their drug-likeness, pharmacokinetics, and physiochemical characteristics, and seven compounds with binding energy lower than flavin (FAD) were identified. Further, molecular dynamics simulation analysis of these seven compounds found that four of them, namely DB12983, DB15688, ZINC084726167, and ZINC254071113 formed stable complexes with the MurB binding site, exhibiting promising inhibitory activity. These compounds have not been mentioned in any other study, indicating their novelty. The study suggests that these four compounds could be promising candidates for treating MTB, but their effectiveness needs to be validated through in vitro and in vivo experiments. Overall, the findings of this study provide new insight into potential drug targets and candidates for combating drug-resistant MTB.

1. Introduction

Tuberculosis is a significant global health concern caused by *M. tuberculosis* and is among the top ten deadliest diseases worldwide. It is the primary cause of death from a single infectious agent and is more prevalent than HIV/AIDS (Global TB Report, 2022). According to the WHO, almost 10 million people worldwide fell ill with TB. The report also states that there were 1.5 million TB-related deaths, in 2020, including 214,000 deaths among people with HIV (Global TB Report, 2022). Despite a consistent decrease in TB cases over time, the estimated number of cases increased by 4.5% from 2020 to 2021, indicating a reversal of the previously observed trend (Global TB Report, 2022). TB account for a substantial number of deaths worldwide, particularly among individuals who are HIV-negative. In 2021, the South-East Asia and African regions, along with India, accounted for 36% of these deaths. When considering both HIV-negative and HIV-positive individuals, these regions accounted for 32% of all TB-related deaths. The number of HIV-TB co-infection cases has been alarmingly increasing over the past decade. Although various treatments are available, the

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evolution of the drug-resistant strain of *M. tuberculosis* highlights the urgent need for new therapeutic approaches to combat multi-drug resistance and HIV-TB co-infection (Konyariková et al., 2020; Kumar et al., 2020). Targeting *Mycobacterium* cell wall synthesis pathways is a promising approach for the development of novel anti-tubercular compounds (Maitra et al., 2019). The sophisticated arrangement of the cell wall structure of *Mycobacterium* species is essential for their survival, pathogenicity, and resistance to various pharmacological therapies (Kumar et al., 2020). The *Mycobacterium* cell wall primarily comprises peptidoglycan (PG), mycolic acid (MA), and arabinogalactan (AG), which together form a complex mAGP. This creates an unusually lipidic and intensely hydrophobic barrier to shield the pathogen from the host's immune system and traditional antibiotics (Daffé and Marrakchi, 2019). The cross-linked materials that compose its mesh-like configuration are NAG and NAM, repeating glycan units that provide cellular structure and integrity while protecting it from osmotic lysis (Kumar et al., 2020). Several research studies have examined the dynamic nature of PG and its various alterations (Maitra et al., 2019). Peptidoglycan provides structural integrity to bacterial cells and is involved in various vital processes, including cell division, cell shape maintenance, and protection against osmotic stress. Inhibition of peptidoglycan biosynthesis disrupts cell wall formation, ultimately leading to bacterial cell death (Zhang et al., 2012). Therefore, targeting enzymes involved in peptidoglycan biosynthesis represents a promising strategy for developing effective antimicrobial agents against MTB.

Bacterial peptidoglycan production is initiated by a set of murine enzymes called Mur enzymes A-F, which catalyze early cytoplasmic steps. Among these enzymes, MurB plays a vital role in the biosynthesis of bacterial cell walls and is an attractive target for drug development. In MTB, the MurB protein comprises three domains and a secondary element characterized by the $\alpha + \beta$ combination. Domain I and II are responsible for FAD binding, while domain III interacts with the substrate. Domain, I span from amino acid residues 21 to 81 and 364 to 369, while domain II covers residues 90 to 244. Similarly, domain III comprises residues 25 to 361, with some residues present at the C-terminus (Eniyan et al., 2018).

The catalytic activity of the enzyme-substrate complex of MurB in *Mycobacterium* is facilitated by a monovalent cation and three essential amino acid residues: Arg 176, Glu 361, and Ser 257 play a crucial role in proton transfer during the second reduction step to an enol intermediate (Daffé and Marrakchi, 2019). Arg 176 and Glu 361 are thought to stabilize the enol intermediate through protonation since the oxygen of the enolpyruvylcarboxylate is close to these residues. While the MurB protein interacts with EP-UDP-GlcNAc and FAD through a total of eleven highly conserved residues, seven of these residues, including Asn 71, Tyr 175, Arg 176, Arg 238, Ser 257, His 324, and Glu 361, are essential for the activity (Daffé and Marrakchi, 2019). Blocking these seven amino acid residues can inhibit the catalytic function of the MurB enzyme. Hence, targeting these residues may provide a promising therapeutic approach to combat *M. tuberculosis* infections.

The process of transforming UDP-N-glucosamine into UDP-N-acetylmuramyl involves a succession of enzymes working together, ultimately resulting in the attachment of five peptides to the latter (Kumar et al., 2020). One such enzyme is MurB, which reduces UDP-N-acetylenolpyruvoylglucosamine, a molecule involved in converting UDP-GlcNAc into UDP-MurNAc. After the formation of UDP-MurNAc, MurB adds a PEP enol pyruvyl moiety and reduces the resulting complex with NADPH into a lactose ether moiety (Abrahams and Besra, 2018; Maitra et al., 2019; Kumar et al., 2020). NamH, a UDP-N-acetyl muramic acid hydroxylase, then hydroxylates UDP-MurNAc to produce UDP-N-glycolylmuramic, a substrate that predominates in the Mtb cell wall (Abrahams and Besra, 2018). Enzymes C to F, which are ligases requiring ATP, catalyze the following steps by adding L-alanine to the carboxyl group of UDP-MurNAc, leading to the production of UPD-N-acetylmuramyl-L-alanine (Maitra et al., 2019). These enzymes share functional and structural similarities, including central ATP binding domains, N-terminal domains that bind nucleotides as substrates, and C-terminal domains that bind amino acids as substrates (Rani et al., 2020). Suppression of enzymes involved in initial peptidoglycan biogenesis leads to cell death via cell wall breakdown and lysis (Chen et al., 2018). However, most antibiotics target the final stages of PG production, neglecting the earlier Mur enzyme-catalyzed steps (Hrast et al., 2014). Studies have suggested that the Mur enzymes could be a promising target for developing new drugs (Abrahams and Besra, 2018; Kumar et al., 2011).

Ligand-based computational virtual screening techniques have greatly aided de novo structural characterization, enabling the identification of potential inhibitors for drug repurposing. SBDD is gaining popularity due to its ability to deliver more precise hits against specific targets at a lower cost than the time-consuming process of random screening. The benefits of theoretical prediction and validation of structural modeling, binding effectiveness, and protein–ligand interaction include the reduction of false positives and the eventual enhancement of specificity in identifying potential hits through in vitro validations (Rožman et al., 2017). In particular, drug repurposing increases the likelihood of discovering an inhibitor by employing previously reported drugs or compounds. Recent studies have identified FDA-approved drugs that could counteract Mtb enzymes MurB and MurE (Rani et al., 2020). Additionally, a screening assay was developed to test several furane-based benzenes-derived compounds against MurE and MurF (Eniyan et al., 2016). Despite this, MurB remains one of the least researched targets for finding potential inhibitors.

In this investigation, a Structure-based methodology was employed to conduct a virtual screening of compounds obtained from three repositories, namely ChemSpider, DrugBank, and the Zinc database. AutoDock Vina was utilized as the docking program, with MurB serving as the protein target. The compounds with the highest binding scores were selected for further analysis. To assess the stability of the protein–ligand interaction, MD simulation (MDS) was performed. MDS is a powerful tool that allows for the study of protein–ligand interactions over a period of time, providing valuable insights into the dynamic behavior of the system. The results obtained from these simulations can help to identify potential inhibitors that may be effective in targeting MurB.

2. Materials and methods

Combining molecular docking and the MDS approach, the process used in this study to identify hit compounds was represented in Fig. 1.

2.1. Retrieval of compounds from repositories

A set of 30,417 compounds were obtained 10,000 from ChemSpider (Pence and Williams, 2010), 9137 from DrugBank (Wishart et al., 2018), and 11,280 from the Zinc database (Sterling and Irwin, 2015). The selection of these compounds was based on their approval, regulatory authorization in context of proven safety and effectiveness and clinical trials. The compounds used in this study were obtained in SDF format. To enable molecular docking studies, these compounds were converted to PDBQT format using the Open Babe tool, which is a widely used tool for chemical file format conversion (O'Boyle et al., 2011).

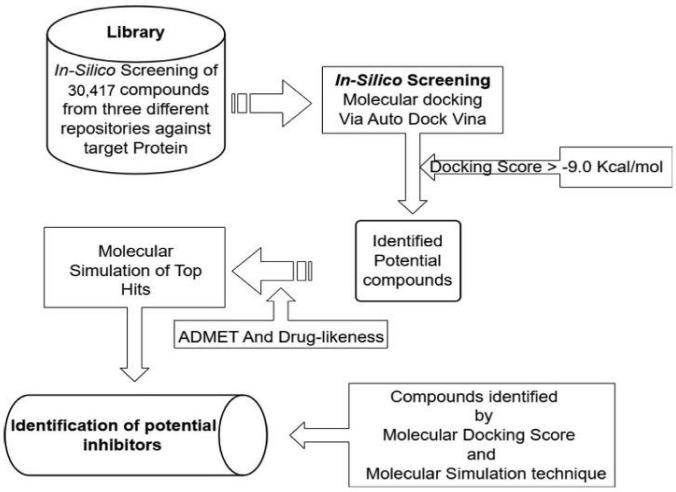


Fig. 1. A diagrammatic representation of methodologies employed in the study for the identification of potent inhibiting compounds targeting the MurB enzyme is depicted herein.

2.2. Protein structure preparation

In this study, we obtained the 2.2 Å MurB crystal structure bound to FAD and K+ (PDB:5JZX) (Fig. 2) from the PDB (Eniyan et al., 2018; Eniyan et al., 2020).

The docking studies were performed using the AutoDock tools (version 1.5.6) (Morris et al., 2009). To ensure the reliability and high quality of the protein structure, redundant dimeric units of the crystal structure, which consisted of six MurB molecules, were removed during the pre-processing stage. The MurB protein was protonated with polar hydrogens that had predetermined Kollman charges.

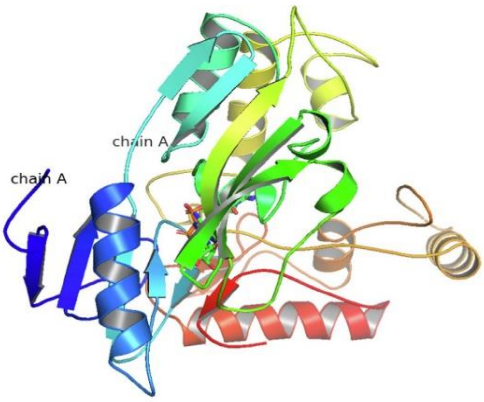


Fig. 2. Three-dimensional (3D) structure of MurB (5JZX).

The PDB was converted to a PDBQT file, which carries information about torsional degrees of freedom and partial charges. The MurB protein was fixed but the side chains and the torsional bonds of the ligand were allowed to move freely. Water and hetatms were removed, and the protein structure was repaired to eliminate any overlapping atoms, unwanted loop sections, and asymmetric side chains. This step also ensured that any missing or overlapping atoms and side chains were straightened out. Overall, the preparation of the MurB protein structure for docking was carried out with meticulous attention to detail to ensure that the resulting protein–ligand interactions were stable and reliable.

2.3. Grid construction and screening

To identify potential ligands that could interact with MurB and induce the desired therapeutic effect of antibacterial activity, virtual screening was performed. Specifically, a molecular docking approach was used to predict the binding orientation of the ligands to the MurB enzyme. The AutoDock vina script (Trott and Olson, 2010) was utilized to screen a large library of compounds (30,417 in total) against the MurB enzyme of *M. tuberculosis*. The docking was carried out using a blind approach, with a grid map set to 100 for X, Y, and Z dimensions, respectively, and a spacing of 0.5 Å. A Lamarckian genetic method was employed, which incorporated free energy and RMSD values to improve the accuracy of the predictions.

Ten docking runs were carried out, each with a population size of 150 and a maximum of 27 K generations. The maximum generation evaluation was set to 2,500 K. The binding affinity of each compound was calculated in terms of kilocalories per mole (Kcal/-mol), and a cut-off of −9.0 Kcal/mol was set as the threshold for screening. The crystal structure of the MurB enzyme and the RMSD values of the docking complexes were taken into account, as well as the inhibition constant (KI).

To visualize the predicted protein–ligand interactions, various tools were used including PyMOL (PyMOL | pymol.org), Protein

Plus, and PLIP (Adasme et al., 2021). These tools allowed for a detailed analysis of the complex interactions between the ligands and the MurB enzyme. The combined prediction from these tools was used to examine the potential interactions between the ligands and the MurB enzyme, to identify compounds with the greatest potential for antibacterial activity.

2.4. MD simulation

A subset of shortlisted ligand-MurB protein complexes was subjected to MDS to further evaluate their potential as antibacterial agents. The complexes were selected based on their docking score and ADMET analysis. To enable significant conformational changes during MDS, the complexes were produced in each direction of the 10 Å X 10 Å X 10 Å buffer of the gradient box. The TIP4P transferable intermolecular potential was used to introduce water molecules into the system.

The MDS was performed using the Desmond version 4.4 module of Schrodinger's Maestro 10.4 (Bowers et al., 2006). Before the MDS, energy minimization was performed in 3,000 steps using the steepest descent technique, followed by the conjugate gradient approach in 5,000 steps with a threshold energy of 120 Kcal/mol. During the MDS, constant pressure was maintained using anisotropic diagonal position scaling on a 0.002 ps time step interval. The system was subjected to a 20 ps NPT reassembly at a target pressure of 1 Atm and a slight increase in temperature from 100 K to 330 K. The Lennard-Jones cut-off value and the Berendsen algorithm were set to 0.2 constant and 9 Å, respectively. The SHAKE ideal constraints were applied to all chemical bonds, including those involving hydrogen atoms (Jorgensen and Tirado-Rives, 1988). The minimized structure's Root Mean Square Deviation (RMSD) was determined by comparing it to the initial structure at 0 ns. This measurement assessed the average variation in atom displacement within a specific frame relative to the initial frame. The RMSD value was computed for every frame throughout the trajectory by using the following equation:

$$RMSD_X = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(r_i'(t_X) - r_i(t_{ref})\right)^2}$$

The system density was kept close to 1 g/cm^3, and all computations were performed using default settings. The OPLS_2005 force field was used for all calculations. Each complex was subjected to MDS for 100 ns intervals using the same parameters. All simulations were performed in triplicate to ensure the reproducibility of the results. The trajectories obtained from the simulations were analyzed using various tools to assess the stability and conformational changes of the complexes over time. The results of the simulations were evaluated in conjunction with the docking scores and ADMET analysis to identify the most promising ligand-MurB protein complexes for further evaluation as antibacterial agents.

3. Results

In the quest for novel therapeutic agents against Mtb, a virtual screening approach followed by MDS was adopted in this study to identify potential inhibitors for the essential MurB enzyme. Based on its significance in Mtb cell biosynthesis, absence in the human body, and documented literature, MurB was selected as the target for this study. A comprehensive screening of compounds from diverse databases was carried out using a Structure-based approach, to identify promising lead compounds for further investigations. Our rigorous methodology enabled the identification of a subset of compounds with a high binding affinity that was subjected to MDS.

3.1. MurB screening and docking analysis

Virtual screening has emerged as a powerful tool in modern drug design, enabling the rapid identification of potential drug candidates with a high affinity for target proteins or nucleic acids. Virtual screening was employed to screen vast chemical databases for their potential biological activity against the MurB enzyme. Through the screening approach, thirty-two potential inhibitors against the MurB enzymes were identified and are shown in Fig. 3.

The figure (Fig. 3) presented displays the results of a screening assay to identify compounds with high binding affinity against the target MurB enzyme. The number of compounds identified showed on X-axis and maximum binding affinity on the Y-axis. Notably, one molecule displayed the highest binding affinity of −13.0 Kcal/-mol, while the lowest binding affinity identifies was −9.70 Kcal/-mol. The binding affinity and inhibition constant values of each compound and other relevant properties are shown in Table 1.

Further analysis of the top thirty-two hits is provided, with a detailed description of their respective binding energies. These results provide valuable insight into exploring binding affinity as a critical parameter in identifying potential inhibitors against target enzymes. Our findings suggest these compounds have the potential to be further studied and optimized as potential inhibitors.

3.2. ADME and Toxicity analysis

In the pursuit of discovering novel therapeutics, identifying compounds with desirable physicochemical and pharmacokinetic properties is a crucial step toward their success as potential therapeutics. To this end, a comprehensive screening approach utilizing cutting-edge tools such as ADME lab 2.0 (Xiong et al., 2021), pkCSM (Pires et al., 2015), and molsoft L.L.C (https://molsoft.com/mprop/) was employed to identify compounds that possess the necessary attributes for drug candidacy.

To assess the suitability of the identified compounds for further identification, a rigorous evaluation of their physicochemical properties was conducted. This included an analysis of key parameters such as MW, lipophilicity, HBD, HBA, and partition coefficient (LogP), among others (Table 1).

In addition, the aqueous solubility, PBP, HIA, BBB, and tumorigenicity of the compounds were also assessed, as these factors can significantly impact the pharmacokinetic profile of drug candidates (Table 2). These evaluations were performed using established methods and criteria, to identify compounds with favorable drug-like properties and a high potential for success in clinical development.

The molecular weight of the compounds ranged from 386.44 to 785.55 g/mol, and the lipophilicity (LogP) ranged from −2.42 to 9.86. In general, for small-drug-like molecules, LogP values can range from about −3 to 6, with most falling within the range of −2 to 4. while the range of the water solubility (LogS) lies from −6.67 to −1.15 mol/L. For most drug-like compounds, LogS values are usually about −5 to 2. Compounds with LogS values below −5 are generally considered to be poorly soluble in water, while LogS values above 2 are typically considered to be highly soluble. To gain deeper insight into the stability and complex interactions between the selected compounds and the target protein, MDS was carried out further.

These findings highlight the meticulous and comprehensive approach taken in evaluating the identified compounds, with a strong focus on key parameters critical to drug discovery and development. This approach is essential in the pursuit of effective treatments for tuberculosis and other diseases caused by bacterial infections and contributes to ongoing efforts to improve global health outcomes.

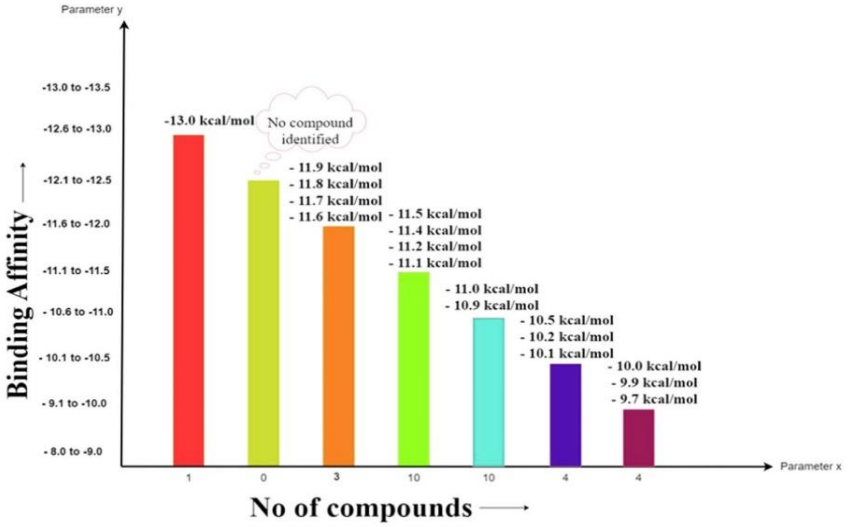


Fig. 3. The calculated free binding energies of the top thirty-two hits interacting with MurB have been determined and are reported herein.

Table 1
A comprehensive evaluation of drug-likeness properties of top thirty-two hits against target MurB.

S. No.	Compound ID	Binding Affinity	Inhibition constant (KI)	Molecular Weight	LogP	H-Bond Acceptors	H-Bond Donors	Drug-likeness Score
1	CSID1438694	−11.1	6.87 nM	417.14	5.96	3	1	−0.26
2	CSID2166135	−11	8.15 nM	428.52	5.94	5	2	−0.53
3	CSID1655442	−10.9	9.94 nM	488.55	7.29	5	1	−0.33
4	CSID2154128	−10.5	19.46 nM	448.95	6.28	5	2	−0.16
5	CSID2165834	−10.2	32.80 nM	414.19	4.52	3	2	−0.17
6	CSID2156566	−10.2	33.62 nM	420.88	4.14	5	2	0.1
7	CSID2140363	−10.1	34.00 nM	397.46	5.13	4	1	0
8	CSID2156621	−10	44.61 nM	502.6	5.53	5	2	0.21
9	CSID3866834	−9.9	54.31 nM	469.79	5.7	3	2	0.66
10	CSID2158441	−9.7	68.21 nM	498.95	5.58	4	2	1.25
11	CSID2156999	−9.7	75.09 nM	386.44	3.59	5	2	−0.2
12	DB15688	−13	55.06 pM	638.37	3.53	8	3	1.3
13	DB12983	−11.8	172.62 pM	514.17	7.42	6	2	−1.06
14	DB14773	−11.5	909.21 pM	478.13	4.68	5	2	0.39
15	DB06229	−11.4	182.65 pM	420.2	4.01	5	0	1.05
16	DB12424	−11.2	153.07 pM	557.22	4.27	5	3	1.11
17	DB15396	−11.1	591.56 pM	532.22	4.64	5	1	1
18	DB15401	−11.1	5.35 nM	579.1	2.13	7	1	0.73
19	DB03461	−11	49.86 nM	743.08	−5.74	20	10	0.66
20	DB11852	−11	440.33 pM	517.11	5.97	4	0	0.08
21	DB08901	−11	19.59 pM	532.22	4.66	5	1	1.06
22	ZINC003975327	−11.9	12.34 nM	582.51	5.76	16	0	−0.99
23	ZINC254071113	−11.6	5.21 nM	775.94	6.05	12	3	0.99
24	ZINC084726167	−11.5	557.57 pM	606.74	4.73	7	0	0.17
25	ZINC008215434	−11.3	6.89 nM	785.55	−2.42	23	6	0.77
26	ZINC095539256	−11.1	4.35 nM	777.88	1.91	13	7	1.29
27	ZINC003934128	−11	711.53 pM	680.76	9.86	6	6	−0.13
28	ZINC004215770	−11	5.93 nM	653.63	1.72	13	5	0.61
29	ZINC003780340	−11	19.28 nM	504.45	5.08	8	4	−1.01
30	ZINC001893112	−10.9	18.00 nM	452.48	4.21	6	2	1.1
31	ZINC003978083	−10.9	56.90 nM	609.74	6.7	6	3	1.11
32	ZINC011616153	−9.5	159.32 nM	612.63	3.62	11	4	1.11

Table 2
ADME and Toxicity analysis of top thirty-two Hits against MurB.

S. No.	Compound ID	PPB (%)	BBB	HIA	Aqueous Solubility (moles/L)	Ames Toxicity	Hepato-Toxicity	Max. Tolerated dose
1	CSID1438694	100.62	−0.04	95.18	−5.55	Yes	Yes	0.56
2	CSID2166135	97.47	0.14	92.73	−4.79	Yes	Yes	0.654
3	CSID 1,655,442	97.36	−0.56	89.63	−4.66	Yes	Yes	0.465
4	CSID2154128	93.78	0.1	93.16	−5	No	Yes	0.551
5	CSID2165834	93.7	−0.08	87.68	−2.89	Yes	No	0.438
6	CSID2156566	94.15	3.99	90.89	−4.7	No	Yes	−0.152
7	CSID2140363	97.05	0	96.81	−6.03	Yes	Yes	0.003
8	CSID2156621	96.56	−0.27	81.73	−2.89	Yes	No	0.438
9	CSID3866834	99.7	4.37	87.12	−5.22	No	Yes	−0.032
10	CSID2158441	95.75	0.101	89.66	−5.46	No	No	0.181
11	CSID2156999	90.29	3.95	92.55	−4.58	No	Yes	−0.169
12	DB15688	81.32	−0.72	87.05	−4	Yes	No	0.438
13	DB12983	95.91	−0.87	78.53	−6.67	Yes	No	0.438
14	DB14773	99.15	−0.23	83.69	−4.81	Yes	No	0.438
15	DB06229	95.56	−0.13	88.71	−4.02	Yes	No	0.438
16	DB12424	93.08	−0.32	80.94	−4.31	Yes	No	0.438
17	DB15396	93.51	0.295	87.5	−4.13	Yes	No	0.438
18	DB15401	95.37	0.62	86.37	−2.7	Yes	No	0.438
19	DB03461	11.77	−5.17	0	−1.15	No	No	0.438
20	DB11852	97.51	0.24	83.96	−6.16	Yes	No	0.438
21	DB08901	93.43	0.39	87.5	−4.22	Yes	No	0.438
22	ZINC003975327	84.03	−2.7	100	−2.89	No	Yes	0.436
23	ZINC254071113	96.9	−2.15	61.57	−3.4	No	Yes	0.52
24	ZINC084726167	83.57	−1.2	92.64	−4.57	No	Yes	−0.214
25	ZINC008215434	67.03	−3.28	22.49	−2.89	No	No	0.274
26	ZINC095539256	89.47	−1.85	50.32	−2.9	No	No	0.335
27	ZINC003934128	98.93	−1.44	100	−2.89	No	No	0.434
28	ZINC004215770	73.46	−1.75	67.67	−3.14	Yes	No	0.176
29	ZINC003780340	77.53	−0.39	100	−2.89	No	No	0.438
30	ZINC003978083	51.37	−2.89	91.33	−2.9	Yes	Yes	0.142
31	ZINC011616153	85.38	−2.058	61.41	−3.46	No	Yes	0.957
32	ZINC001893112	96.67	3.37	92.69	−4.28	No	Yes	0.346

Using this rigorous screening criteria, a total of thirty-two compounds were identified as potential candidates. Further refinement based on the maximum lower range of binding energies between −11.0 and −13.0 Kcal/mol, resulted in the selection of seven compounds that exhibited exceptional binding properties. These seven compounds were subsequently subjected to MDS to evaluate the stability of their interactions.

3.3. Compounds interactions with protein residues

The top seven hits with the binding affinity ranging from −11.0 to −13.0 Kcal/mol were selected for further analysis. Specifically, the interacting residues of the MurB enzyme with these seven compounds were examined to gain insight into their potential mechanism of action and binding pocket. All selected compounds fit well within the target MurB enzyme's cavity and their postures and interactions were analyzed (Figure S1). Of note, residue Ile 127 was found to be a frequent residue in the pocket where inhibitors against MurB bind, indicating its involvement in the binding of many other molecules.

Detailed analysis of selected compounds revealed distinct hydrophobic and hydrogen bond interactions with specific residues of the target enzyme. For instance, the compounds from ChemSpider with CSID1438694 (Fig. S1A) showed nine hydrophobic interactions and two hydrogen bond interactions, while the compound from DrugBank with DBID12983 (Fig. S1C) interacted with five hydrophobic residues and three hydrogen bond interactions with one π-stacking and one π-cation interaction. The second compound CSID2166135 (Fig. S1B) was found to interact with several specific amino acid residues of the target MurB enzyme. Specifically, it showed interactions with five hydrophobic residues, including threonine at position 26, isoleucine at 127, proline at 128, alanine at 141, and leucine at position 245. In addition, it was found to interact with four amino acid residues via hydrogen bonds, specifically serine at position 70, arginine at 238, serine at 257, and glutamic acid at position 361.

Compound DBID15688 depicted in Fig. S1D manifests an extensive network of molecule interactions comprising thirteen contacts, among which six involve hydrophobic interactions and seven engage in hydrogen bonding. The hydrophobic contacts consist of Ile 127 (two contacts), Val 139, Ala 141, Tyr 175, and Tyr 210. The hydrogen bonding interactions are established with Arg 176, Tyr 210, Ser 257, Asn 261, Glu 302, Ala 325, and Glu 361. This comprehensive interaction profile delineates the intricate interplay of hydrophobic and hydrogen bonding forces governing the binding of DBID15688 to its target, thereby illuminating its potential therapeutic applications.

The compound from Zinc Database, ZINC003975327 (Fig. S1E), interacted with three hydrophobic residues with Tyr 175 (two contacts), and Val 263 and two hydrogen bond interactions with Tyr at position 175 and Thr at 177. The compound ZINC084726167 (Fig. S1F) interacted with three hydrophobic residues and six hydrogen bond interactions. The identified hydrophobic interaction involves Tyr 210, Tyr 287, and Ala 296, while the hydrogen bond interactions are mediated by Tyr 175, Arg 176, Ser 257, Asn 261, and His 324. Additionally, a salt bridge interaction is formed between Glu 361 and the proteins binding partner. Notably, some residues were common among the compounds, suggesting a common binding pocket for these inhibitors. For example, residues Tyr 210 and Tyr 175 were found to have interactions in two compounds, including those with compounds ID DB15688 (Fig. S1D), and ZINC084726167 (Fig. S1F) further supporting the notion of a shared binding pocket.

ZINC254071113 (Fig. S1G) establishes a network of eight molecules interactions with their target enzyme, featuring three hydrophobic and four hydrogen bond interactions with one π-

stacking. Specifically, the hydrophobic contacts involve Tyr 210, Pro 283, and Pro 287, while the hydrogen bonding contacts are established with Asn 261, Val 284, His 286, and Gly 298. These results offer a valuable framework for the design of novel MurB inhibitors with enhanced potency and selectivity.

3.4. MDS of MurB with ligands

The results of the MD trajectory analysis of seven ligands, selected based on their high binding affinity during molecular docking, were examined to determine their potential as inhibitors of MurB protein in tuberculosis drug development. The analysis revealed that four of the seven ligands formed stable complexes with MurB, indicating strong protein–ligand intermolecular interactions (Fig. 4). In particular, CSID1438694 (Fig. 4A) complex demonstrated weak stability and binding within the initial 15 ns, with slight diffusion of the ligand observed between 15 and 20 ns with a maximum deviation of 3.6 Å. A synchronous fluctuation between the protein and ligand was observed in the 20–30 ns timeframe. Although the ligand diffused away from the MurB protein between 40 and 80 ns, it showed weak binding affinity and an RMSD of the protein at 3.2 Angstrom, and the ligand fluctuated sharply at 3.6 Å in the last time frame (80–100 ns). Overall, CSID1438694 exhibited weak binding with MurB protein.

Ligand CSID2166135 (Fig. 4B) did not demonstrate promiscuous binding with MurB protein, showing diffusion away from the protein's binding site during the 100 ns timeframe, with only slightly weak binding observed at 40 ns. Ligand DB12983 (Fig. 4C) exhibited strong binding with MurB protein during the 10–60 ns timeframe, but later diffused away, with RMSD ranging between 2.2 Å and 3.8 Å, and showed more aberrant fluctuations than the protein during the 20–50 ns timeframe. The second compound from the drug Bank DB15688 (Fig. 4D) exhibits an attractive interaction between the receptor and the ligand. The ligand shows diffusion behavior during the initial 0–35 ns of the simulation, but subsequently, it forms consistent and stable binding interactions with the receptor for the remainder of the simulation times. These findings suggest that DB15688 has the potential to be an effective inhibitor for the receptor of interest. Ligand ZINC003975327 (Fig. 4E) did not exhibit consistent interactions with the target protein, as per RMSD values ranging from 0.5 Å to 3.9 Å for the ligand. Ligand ZINC084726167 (Fig. 4F) undergoes initial diffusion behavior during the initial 0–20 ns of the simulation, followed by a period of consistent and stable binding interaction with the receptor between 20 and 80 ns. Once the ligand establishes a stable binding interaction with the receptor, it forms a complex that can maintain its stability for a prolonged period. However, after 80 ns, the ligand shows a rapid and pronounced dissociation from the receptor.

Ligand ZINC254071113 (Fig. 4G) diffused away from the target after 80 ns timestep, however, a resonance between the alpha carbon atom of the protein backbone and the atomic coordination of the ligand was observed between 10 and 80 ns. Therefore, ligands DB12983 (Fig. 4C), DB15688 (Fig. 4D), ZINC084726167 (Fig. 4F), and ZINC254081113 (Fig. 4G) exhibited stable binding with the MurB protein, with RMSD values ranging between 2.1 Å and 3.6 Å. These findings demonstrate the stability of protein and four out of seven molecules that made the shortlist after MD analysis. The identified compounds exhibit promising inhibition of Mtb and may have a unique mode of action. They could be used as a starting point for chemical modification in medicinal chemistry to create a higher-affinity scaffold with improved inhibitory action.

Our docking and MDS analysis revealed a set of ligands, namely DB12983, DB15688, ZINC084726167, and ZINC254071113, as inhibitors of the MurB enzyme in MTB. Our study revealed that these compounds exhibited consistent and stable interactions with the MurB, displaying very good binding affinity. Our findings indicate that these compounds possess the potential to act as potent inhibitions of Mtb by interacting with the MurB enzyme. Table 3 provides detailed information on the interacting residues of the MurB-ligand complexes, including information on their structure characteristics, hydrophobic and hydrogen bond interactions, and more. Our analysis demonstrates that these four hits remained

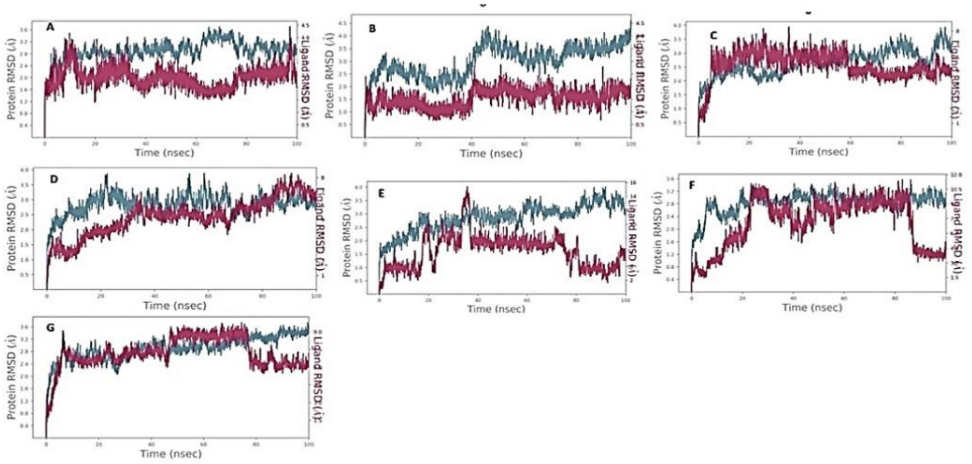
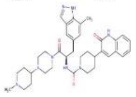
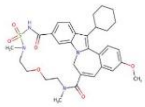
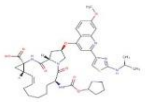


Fig. 4. RMSD between MurB of *M. tuberculosis* and selected ligands from various libraries. The ligands shown are A-CSID1438694, B-CSID2166135, C-DB12983, D-DB15688, E-ZINC003975327, F-ZINC084726167, G-ZINC254071113.

Table 3
Top four hits identified Against Target MurB enzyme and their interacting residues.

Hit ID	Compound ID and Structure	G-Score (Kcal/mol)	H-Bond	Hydrophobic Interactions
Hit 1		−11.8	Tyr 210 Asn 261 Lys 294	Glu 145 Asn 261 Tyr 287 Pro 288 Ala 296
Hit 2		−13.0	Arg 176 Tyr 210 Ser 257 Asn 261 Glu 302 Ala 325 Glu 361	Ile 127 Ile 127 Val 139 Ala 141 Tyr 175 Tyr 210
Hit 3		−11.5	Tyr 175 Arg 176 Arg 176 Ser 257 Asn 261 His 324	Tyr 210 Tyr 287 Ala 296
Hit 4		−11.6	Asn 261 Val 284 His 286 Gly 298	Tyr210 Pro283 Pro287

stable within the active site of UDP-N-acetylenolpyruvoylglucosa mine reductase in *M. tuberculosis* and exhibited consistent interactions throughout the simulation.

The table (Table 3) likely summarizes the results of molecular docking or MD study that aimed to identify potential ligands that could bind to the MurB enzymes. The table may also list the specific residues in the MurB enzymes that were found to be involved in ligand–protein interactions. This information can provide insight into the mechanism of binding and help in designing more effective inhibitors.

4. Discussion

The emergence of drug resistance has become a major concern in the fight against infectious diseases, necessitating the search for new compounds with unique mechanisms of action. The development of novel molecules across different classes entails a series of intricate processes, which include the discovery of new compounds with distinct mechanisms of action, the identification of potential inhibitors, and the chemical modification of existing drugs. These processes are crucial in the pursuit of effective and safe therapeutic agents for various ailments. Several approaches have been extensively utilized to discover potential inhibitors, such as high throughput screening, whole-cell-based screening, and combinatorial synthetic chemicals. These techniques have facilitated the identification of a diverse array of Mur enzyme inhibitors sourced from different organisms and a plethora of antitubercular scaffolds (Hrast et al., 2013; Hrast et al., 2014). Currently, these inhibitors are being assessed at varying stages of clinical trials, suggesting their promising therapeutic properties (Loerger et al., 2013).

Bedaquiline represents a successful outcome of using target-based high-throughput screening to identify an antitubercular drug that hinders the MTB's ATP synthase enzyme (Kundu et al., 2016). The availability of small-molecule libraries and the progress in computational techniques have presented more opportunities for discovering novel chemical scaffolds that target specific proteins. However, despite these advancements, the effectiveness of TB drug discovery research remains hindered by the absence of experimental validation of in silico hits and the challenge of translating in-vitro activity into mycobactericidal activity and vice versa (Eniyan et al., 2020).

In recent times, Mur enzymes found in Mtb have gained significant attention as a potential drug target owing to their indispensable role in the survival of the pathogen (Kouidmi et al., 2014; Jukič et al., 2019; Yang et al., 2006). Readers are directed to a comprehensive review by Hrast and colleagues which highlights an array of broad-spectrum chemical inhibitors that effectively target bacterial Mur ligases (Hrast et al., 2014). This review delivers into the mechanistic underpinnings of these inhibitors and the structural features that facilitate their binding to MurB. Several inhibitors targeting MurB have been documented in the literature (Bronson et al., 2003; Francisco et al., 2004; Kutterer et al., 2005). Additionally, the significance of MurB as a target in Mtb is supported by scientific studies. For example, a study by Eniyan and colleagues, provided insight into the structure and function of MurB, emphasizing its significance in the peptidoglycan biosynthesis pathway (Eniyan et al., 2018). Another research study by Rani and colleagues, aimed to identify potential drugs that could inhibit MurB enzymes, suggesting MurB is a potential target of MTB (Rani et al., 2020). A research study by Kumar and colleagues contributes to our understanding of the structure and function of the MurB enzyme and provides valuable insights for future drug discovery efforts targeting peptidoglycan biosynthesis (Kumar et al., 2011). Furthermore, other research studies by Bronson and colleagues have highlighted the essentiality of MurB in Mtb, finding presents opportunities for the development of novel antibacterial agents that can effectively target the MurB enzyme and potentially address antibiotic-resistant bacterial infections (Bronson et al., 2003). According to the studies conducted by Kumar and colleagues, the compound under investigation serves as a substrate for the MurB, which function as a reductase and exerts its catalytic activity on the substrate. Notably targeting the MurB enzyme presents an opportunity for selective inhibition, as it is not present in

the Human system. This characteristic renders the MurB a promising candidate for identifying inhibitors with potential therapeutic implications (Kumar et al., 2020).

To identify inhibitors that are specific to the MurB enzyme, various compound repositories were screened in this study. The MurB enzyme was selected for the screening process as crystal structures of this enzyme in Mtb have been previously solved. We used 30,417 compounds from three different databases to screen for MurB inhibitors. From the initial screening, the top thirty-two compounds were selected based on their binding affinity, drug-likeness, and ADMET properties. To assess the stability and interactions of the compounds with the MurB enzymes, MDS was performed. Among the screened compounds, DB12983, DB15688, ZINC084726167, and ZINC2540741113 emerged as the most favorable candidates based on their strong binding affinity, stable conformations, and robust interactions with the key residues. The binding affinity of these compounds, as indicated by their docking score of −11.8 Kcal/mol, −13.0 Kcal/mol, −11.5 Kcal/mol, and −11.6 Kcal/mol, respectively, suggests their potential as potent inhibitors of MurB.

Analysis of the PLIP revealed that these compounds interacted strongly with key residues, namely Tyr 175, Asn 261, Tyr 210, Arg 176, and Ser 257. Tyr 175 were found to be common in multiple ligands including DB12983, DB15688, and ZINC084726167 indicating their essential role in the binding of these compounds. Ser 257 is found to have common interaction in DB15688 and ZINC084726167. Additionally, Asn 261 and Tyr 210 were also found to be common in DB12983, DBID15688, ZINC254071113, and ZINC084726167, suggesting their critical role in the binding of these inhibitors to MurB. The results of this study revealed that four potential compounds interact with previously reported residues in the active site MurB of *M. tuberculosis*. These residues have been previously shown to play a critical role in the enzyme's activity and are considered important targets for inhibition by Daffé and Marrakchi (Daffé and Marrakchi, 2019). The fact that the potential compounds identified in this study interact with these key residues suggests that they may have the potential to inhibit MurB activity and serves as promising lead compounds for further optimization.

These compounds can be used as a basis for further chemical alterations and refinements aimed at enhancing their inhibitory effects and creating scaffolds with greater affinity. The MDS provided valuable insight into the stability and interactions of the compounds with the MurB enzyme, which could aid in the development of more effective inhibitors of MTB.

This investigation presents a successful approach that combines a Structural-based screening with MDS to efficiently identify potential inhibitors for further development. Our results demonstrate the potential of this methodology for high-throughput screening of larger compound libraries, providing a valuable tool for drug discovery research.

5. Conclusion

In conclusion, our study has identified four promising compounds, DB12983, DB15688, ZINC084726167, and ZINC254071113, that can effectively inhibit the MurB enzyme, presenting a potential strategy for inhibiting the initial stage of PG biosynthesis in *M. tuberculosis*. These compounds have demonstrated excellent binding and stable conformation with the MurB enzyme, and have the potential to serve as effective antimycobacterial agents, including drug-resistant strains. Further experimental validation of these compounds as potential inhibitors is warranted, and may pave the way for the development of novel antimicrobial therapies for tuberculosis. The current study represents a significant breakthrough in the field of tuberculosis research, as we are the first to report the efficacy of these compounds against drug-resistance *Mycobacterium tuberculosis*. This finding is particularly noteworthy because drug resistance is a major barrier to the treatment of tuberculosis, which has caused significant challenges in global health. Our study provides a potential solution to this problem by identifying new compounds that can effectively inhibit the MurB enzyme, an essential component of tuberculosis cell wall synthesis. By targeting this enzyme, our compounds have the potential to overcome the resistance mechanism of tuberculosis and serve as an effective therapy for drug-resistant strains. Additionally, the study may have employed innovative approaches such as computational approach, high-throughput screening, or structure-based drug design to identify these potential inhibitors. These promising results open up new avenues for the development of novel antimicrobial therapies and have significant implications for the future treatment of tuberculosis.

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CRediT authorship contribution statement

Ankit Verma: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Vijay Kumar:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Bindu Naik:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Javed Masood Khan:** Writing – review & editing, Writing – original draft, Resources. **Pallavi Singh:** Writing – review & editing, Methodology. **Per Erik Joakim Saris:** Writing – review & editing, Writing – original draft. **Sanjay Gupta:** Writing – original draft, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103730.

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A research paper entitled “Screening and molecular dynamics simulation of compounds inhibiting MurB enzyme of drug-resistant *Mycobacterium tuberculosis*: An *in-silico* approach”

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Review

Revolutionizing Tuberculosis Treatment: Uncovering New Drugs and Breakthrough Inhibitors to Combat Drug-Resistant *Mycobacterium tuberculosis*

Published as part of ACS Infectious Diseases virtual special issue "One Health and Vector Borne Parasitic Diseases".

Ankit Verma, Bindu Naik, Vijay Kumar,* Sadhna Mishra, Megha Choudhary, Javed Masood Khan, Arun Kumar Gupta, Piyush Pandey, Sarvesh Rustagi, Barnali Kakati, and Sanjay Gupta

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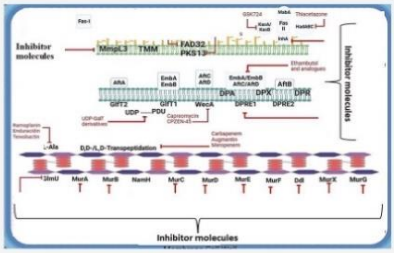
ABSTRACT: Tuberculosis (TB) is a global health threat that causes significant mortality. This review explores chemotherapeutics that target essential processes in *Mycobacterium tuberculosis*, such as DNA replication, protein synthesis, cell wall formation, energy metabolism, and proteolysis. We emphasize the need for new drugs to treat drug-resistant strains and shorten the treatment duration. Emerging targets and promising inhibitors were identified by examining the intricate biology of TB. This review provides an overview of recent developments in the search for anti-TB drugs with a focus on newly validated targets and inhibitors. We aimed to contribute to efforts to combat TB and improve therapeutic outcomes.



KEYWORDS: *Mycobacterium tuberculosis, drug resistance, novel targets, peptidoglycan biosynthesis, arabinogalactan biosynthesis, mycolic acid biosynthesis, chemical inhibitors, drug development*

Tuberculosis (TB) is a highly lethal disease that surpasses human immunodeficiency virus (HIV) and malaria in terms of mortality. It is caused by various species of the *Mycobacterium* complex, including *Mycobacterium tuberculosis* (MTB) and other genetically associated species. MTB belongs to Actinomycetales and Corynebacterial suborders within the Gram-positive Actinobacteria phylum.[1] TB patients can be categorized into three groups: latent TB infection (LTBI), active TB, and subclinical TB, each with distinct characteristics and treatment options.[2] Various diagnostic methods, including sputum smear microscopy, Xpert MTB/RIF, and Hain line test, have facilitated the rapid identification of the *Mycobacterium* complex (MTBC) responsible for TB.[3] However, the confirmation of drug-resistant TB diagnosis solely on the basis of these factors remains uncertain.

The global fight against TB has brought together organizations, such as the World Health Organization (WHO), multiple governments, pharmaceutical companies, nongovernmental organizations (NGOs), and academic institutions. Collaborative efforts through partnerships such as the Global TB Alliance, STOP-TB partnership, TB drug accelerator, and treatment of TB aim to combat the disease.[4] Scientists face significant challenges in this battle, including understanding MTB biology, discovering new TB targets, developing patient-friendly drug regimens for individuals with HIV and diabetes, shortening treatment duration, improving cost-effectiveness, advancing diagnostics, and creating novel drugs.[5] Addressing these challenges is crucial in TB diagnosis and treatment.

To effectively treat *Mycobacterium tuberculosis* infections, healthcare providers typically use a combination of antibiotics to prevent drug resistance and increase the likelihood of successful treatment.[2] The most common drugs used in the treatment of TB include Isoniazid (INH): This drug is a cornerstone of TB therapy and is effective against actively dividing bacteria. Rifampin (RIF): it is another essential drug in TB treatment, targeting both actively dividing and dormant bacteria.

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Pyrazinamide (PZA): PZA is particularly effective against dormant bacteria in acid-fast bacilli. Ethambutol (EMB) is often included to reduce the risk of drug resistance. Streptomycin or Aminoglycosides: These drugs may be used in cases of drug resistance or severe TB.[2,6]

TB treatment usually involves two phases: an initial intensive phase and a continuation phase. During the intensive phase, multiple drugs are administered simultaneously to rapidly decrease the bacterial load. The continuation phase then focuses on eliminating any remaining bacteria. This sequential approach helps reduce the risk of resistance.[2] The treatment of TB is continuously evolving, with researchers exploring novel drug combinations and approaches. Some plans and promising developments include (1) Shorter Treatment Regimens: Efforts are underway to develop shorter, more patient-friendly treatment regimens to improve treatment adherence and completion rates.[5] (2) New Drug Candidates: Several new drugs, such as bedaquiline and delamanid, have been introduced and are being investigated for their effectiveness against drug-resistant TB.[5] (3) Combinations with Host-Directed Therapies: Researchers are exploring the combination of anti-TB drugs with host-directed therapies to enhance the immune system's ability to combat TB.[5] (4) Pharmacokinetic Optimization: Studies are focused on optimizing drug dosing and administration to maximize efficacy and minimize side effects.[5] (5) Drug Resistance Management: Ongoing research aims to develop strategies for the management of drug-resistant TB, including new combinations and treatment approaches.[5,7]

Exploring targets and their inhibitors within the *Mycobacterial* cell wall pathways constitutes a crucial endeavor in the realm of tuberculosis (TB) drug discovery and therapeutic development. *Mycobacterium tuberculosis*, the causative agent of TB, possesses a unique and complex cell wall architecture, primarily composed of mycolic acids, peptidoglycans, and arabinogalactan, which is instrumental for its survival and pathogenicity.[5,8] This intricate structure serves as a barrier to host immune defenses and plays a pivotal role in resisting antibiotics. Therefore, targeting specific components and enzymes involved in *Mycobacterial* cell wall biosynthesis and maintenance represents a promising strategy to combat TB.[8] The importance of targeting these pathways lies in the potential to disrupt the integrity of the cell wall, rendering the bacterium susceptible to the host immune system and existing antibiotics.[5] Moreover, such targeted therapies could mitigate the emergence of drug-resistant strains, addressing a pressing global health concern. Consequently, the exploration of *Mycobacterial* cell wall pathways and the development of inhibitors against these targets offer a compelling avenue for advancing TB treatment and control.[5,8]

Addressing TB urgently is critical to prevent loss of life, contain the spread of the disease, reduce drug resistance, mitigate economic and societal impacts, and work toward global health security and the eventual elimination of TB as a major public health threat. It requires a coordinated effort from governments, healthcare systems, and international organizations to effectively combat this infectious disease.[6,7]

This review aims to provide an overview of recent developments in the search for anti-TB drugs with a specific emphasis on newly validated targets and inhibitors. By examining the intricate biology of TB, we have identified emerging targets and promising inhibitors. In doing so, we shed light on the evolving landscape of TB research and drug discovery, highlighting the critical areas of focus in our pursuit of more effective treatments.

MYCOBACTERIUM TUBERCULOSIS

In 2021, the global burden of TB has increased, with 10.6 million people being diagnosed, representing a 4.5% increase compared with 2020. This has reversed the declining trend observed over the past few years.[6] The incidence rate of TB also saw a 3.6% increase after two decades of a consistent decline. The highest number of cases was reported in Southeast Asia (45%), Africa (23%), and the Western Pacific region (18%). HIV coinfection poses a significant challenge in the fight against TB, with 6.7% of all TB cases occurring in HIV-infected individuals.[7] Poverty, limited vaccine effectiveness, complex diagnostics, and challenges in drug treatment adherence complicate TB control. Addressing these challenges is crucial for improving public health outcomes and reducing the impact of TB.[8] Advancements in anti-TB drugs have evolved, with the first clinical experiment conducted in 1948 and the subsequent development of drugs targeting drug-resistant strains.[9]

To achieve the Millennium Development Goals, the WHO implemented directly observed therapy for treating tuberculosis (DOTS) and Stop TB initiatives from 2000 to 2015. Subsequently, the End TB strategy (2016−2035) was aimed at global TB elimination. However, significant challenges persisted in 2016, including cases of drug-resistant tuberculosis (DR-TB). Additionally, 6% of multidrug-resistant tuberculosis (MDR-TB) cases are classified as extensively drug-resistant tuberculosis (XDR-TB) owing to their heightened resistance to certain drugs.[10] Many countries face difficulties in achieving the goals set by the End TB program, mainly due to undervaluation, underdetermination, and issues with the TB drug care cascade.[11] To address these challenges, TB prevention should focus on programmatic and clinical perspectives. Programmatic approaches should emphasize improved drug utilization and adherence to tailored regimens, whereas clinical strategies should enhance surveillance, manage drug-resistant cases, and evaluate existing or novel medications.[12] Lengthy and complex medication regimens pose a major hurdle to TB treatment by affecting adherence and causing adverse effects. Additionally, the intersection of HIV and TB complicates the drug interactions between anti-TB agents and anti-retroviral treatments. Overcoming these challenges is crucial for effective TB control and treatment.

THE EMERGENCE OF DRUG RESISTANCE

MTB can adapt to the human immune system, enter a dormant state, evade immune responses, and persist within the host. During dormancy, MTB gains resistance through Reactive Nitrogen intermediate (RNI) production, altering host immune processes. Reactivation from dormancy is facilitated by resuscitation-promoting factors and peptidoglycan-hydrolyzing enzymes regulated by the dormancy survival regulator (DosR) regulon system.[13] MTB's transition of MTB between respiring and nonrespiring environments coupled with its persistence under adverse conditions contributes to its high infectiousness.

Drug resistance is a major challenge in TB control, because environmental factors induce genetic alterations that reduce medication effectiveness and promote survival under extreme conditions. This survival mechanism can lead to MDR and increased mutagenesis in the presence of bactericidal antimicrobials.[14] TB drug resistance is classified as multidrug resistance (MDR-TB), extensive drug resistance (XDR-TB), or total drug-resistant tuberculosis (TDR-TB or XXDR-TB).

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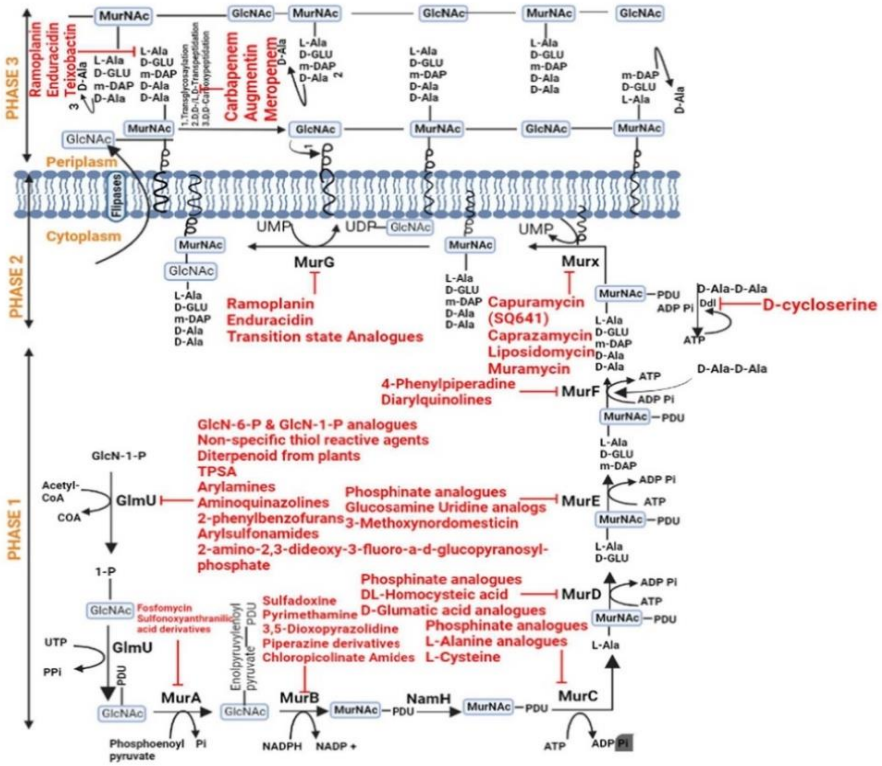

Figure 1. Biosynthesis of peptidoglycan with an arrow pointing in the direction of the target; inhibitors for various enzymes in Phases I, II, and III are displayed in red text.

MDR-TB is prevalent worldwide and renders first-line drugs ineffective, whereas XDR-TB and TDR-TB are less common.

Drug resistance poses a significant global challenge in TB treatment. MTB has developed various mechanisms to resist the effects of anti-TB medications. These mechanisms include clonal interference, compensatory development, cell envelope impermeability, efflux pumps, epistasis, phenotypic drug tolerance, target mimicry, and drug degradation and modification.[15] Intrinsic resistance, inherent to MTB, is observed, owing to its complex cell wall structure and the presence of β-lactamase enzymes. Prolonged exposure to low drug doses can trigger carrier protein overexpression, leading to phenotypic and hereditary resistance.[16] MTB has evolved the ability to adapt to the cytotoxicity of antibiotics and other drugs, which has accelerated the development of intrinsic drug resistance.[17] Acquired drug resistance occurs through the chromosomal transformation of drug-targeted genes during treatment, resulting in the transmission and expansion of resistant strains. Factors such as extended treatment, sporadic medication consumption, and poverty contribute to the development of acquired drug resistance.[16]

THE *MYCOBACTERIUM* CELL WALL

Mycobacterial cell walls of *Mycobacteria* consist of peptidoglycan (PG), lipopolysaccharide (LPS), and an outer layer that contains mycolic acid (MA). The PG layer (Figure 1) provides rigidity, integrity, and shape to cells.[18] PG in *Mycobacteria* is composed of *N*-acetylglucosamine and muramic acid residues linked by β (1−4) bonds. Mur ligases (MurC/D/E/F) produce the pentapeptide L-alanyl-D-isoglutaminyl-meso-diaminopimelyl-D-alanyl-D-alanine, which is acylated on the muramic acid component by polysaccharide strands.[19] Cross-linkages in PG are formed by penicillin-binding proteins (PonA1 and PonA2) and transpeptidases (L, D, and LdtMt1−5).[18]

Arabinogalactan (AG) in *Mycobacteria* is a polysaccharide composed of arabinose and galactose sugars (Figure 2). Its biosynthesis involves the creation of a linker that binds to PG. This process includes enzymatic steps mediated by WecA, WbbL, GlfT1, GlfT2, AftA, EmbA, and EmbB.[5] AG plays a crucial role in the structural stability of the mycelial-arabinogalactan-peptidoglycan complex by retaining mycolic acids in place. Understanding its biosynthesis provides insights into targeted interventions against *Mycobacterial* infections.[19]

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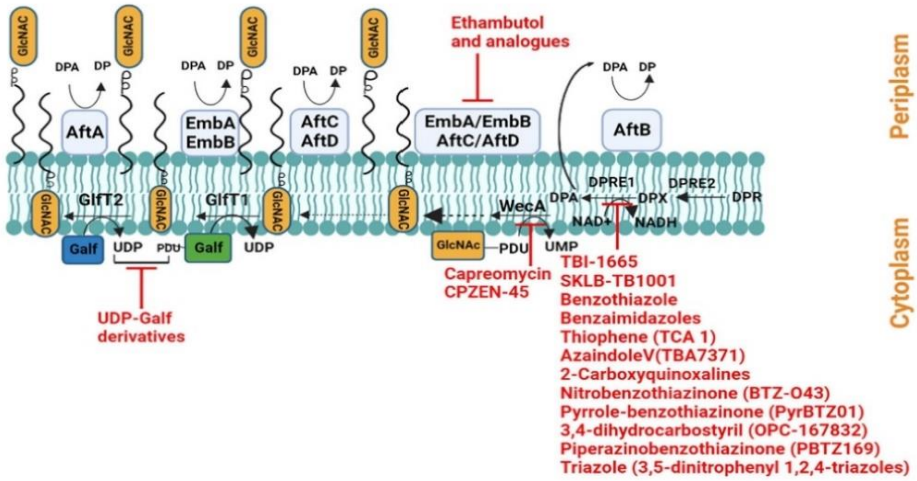

Figure 2. Biosynthesis of Arabinogalactan with an arrow pointing in the direction of the target, inhibitors for several enzymes involved in the cytoplasm, and periplasm is shown in red text.

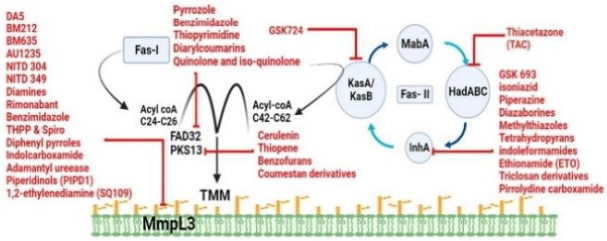

Figure 3. Biosynthesis of mycolic acid with an arrow pointing in the direction of the target, inhibitors for several targeted enzymes are shown in red text.

MA, the vital component of the MTB cell wall, affects the acid-fast staining, virulence, viability, and permeability (Figure 3). Trehalose mono/dimycolates (TMM/TDM) and glucose monomycolate can be produced by coupling mycolic acids to other saccharides or by finding them in a free state. MA binds to the arabinose component of the AG complex.[20]

■ PEPTIDOGLYCAN BIOSYNTHESIS AND POTENTIAL TARGETS

Inhibiting cell wall biosynthesis, particularly PG formation, is a promising approach for the development of bactericidal drugs against MTB. The unique composition and structure of the PG layer in MTB make it an attractive target for drug repurposing and the development of novel drugs. PG biosynthesis in MTB occurs in three stages and involves enzymes located in different cellular compartments. Phase I involves the synthesis of cytoplasmic precursors; Phase II involves the translocation of membrane-bound precursors over the periplasm; and Phase III involves precursor polymerization and peptide cross-linking (Figure 1). Understanding these biosynthetic pathways is crucial for identifying enzymatic targets and developing pharmacological inhibitors.[21] GlmU, an enzyme with acetyltransferase activity in MTB, is considered a potential target because of its absence in humans. Inhibitors of bacterial Mur ligases, which are involved in precursor polymerization and peptide cross-linking, have also been identified. One-pot assays and computational methods have been employed to identify and understand the binding of inhibitors to these enzymes.[22]

Certain antibiotics, such as cycloserine and capreomycin-based compounds, disrupt PG production by binding to specific targets involved in the process. Antibiotics that bind to Lipid II, a precursor molecule, have also shown efficacy in inhibiting TB.[23] Among these potential targets, glutamate racemase, which is encoded by MurI, has emerged as a promising candidate. This enzyme plays a crucial role in the initial stages of PG synthesis and has additional functions, including sequestering DNA gyrase enzymes. Glutamate racemases are widely conserved in bacteria and lack eukaryotic counterparts, making them appealing targets for drug development.[24]

MurA Inhibitors. MurA, an enzyme involved in PG synthesis, is a potential therapeutic target for tuberculosis

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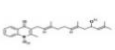
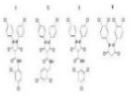
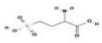
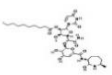
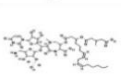
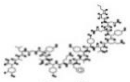
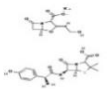
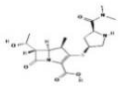
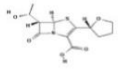
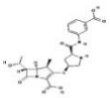
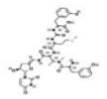
Table 1. Inhibitors Targeting the Peptidoglycan Biosynthesis Pathway

Compound /Inhibitor	Chemical class	Structure	Cell wall components inhibited	Ref
Fosfomycin	Phosphonic		MurA	26
Aurachin RE	Prenylated quinoline		MurA	32
3,5-dioxopyrazolidine	Pyrazolidines		MurB	26
DL-homocysteic acid	Non-proteinogenic alpha-amino acid		MurD	26
CS (D-cycloserine)	D-cycloserine		ddlA	33
SQ641	Capuramycin		MurX	5
Caprazamycin	Natural product		MurX	23
Liposidomycin	Lipopeptides		MurX	23
Muramycin	Nucleoside antibiotics		MurX	23
Ramoplanin	Glycolipodepsipeptide		MurG	26
			L-Ala	34
Enduracidin	Cyclic polypeptide		MurG	26
			L-Ala	34
Teixobactin	Cyclodepsipeptide		L-Ala	34

Compound /Inhibitor	Chemical class	Structure	Cell wall components inhibited	Ref
Carbapenem	β -lactam		D, D-/L, D-Transpeptidation	35
Augmentin	Penicillin’ s		D, D-/L, D-Transpeptidation	35
Meropenem	Carbapenem		D, D-/L, D-Transpeptidation	35
Faropenem	β -lactam antibiotics		D, D-/L, D-Transpeptidation	35
Ertapenem	Carbapenem β -lactam		D, D-/L, D-Transpeptidation	35
Friulimicin B	Oligopeptides		C55-PP	36
Mureidomycin A	Peptidylnucleoside		MurY	37
Teixobactin	Lipo-peptide		Lipid II	5
Sanfetrinem	The third generation of cephalosporins		Inhibit peptidoglycan synthesis. Exact target unknown	38
GlcN-1-p analogs	Derivatives of glucosamine		GlmU	5
Phosphinate analogs	Phosphonates		MurD and MurE	26
D-Glumatic acid analogs	Amino acids		MurD	26
3-Methoxynordomesticin	Coumarins	NA	MurE	26
Diarylquinolines	Quinolines		MurF	26
4-Phenylpiperadine	Piperidine		MurF	26
Pacidamycins				
Napsamycins	Uridyl peptide	NA	MurY	26

treatment. However, the clinically approved MurA inhibitor fosfomycin is ineffective against *Mycobacterium* owing to structural differences in its active site (Table 1). Inhibition of the transferase enzyme MurA is a promising treatment strategy.[25] Various compounds, including peptidomimetics and derivatives of sulfonoxyanthranilic acid, have demonstrated inhibitory effects on the MurA enzyme of MTB (Figure 1). Studies have shown that peptidomimetic substances and sulfonoxyanthranilic acid derivatives exhibit promising inhibitory activity against MurA.[26] Kumar et al. identified six

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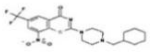
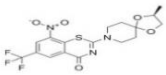
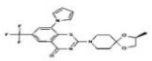
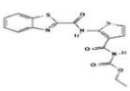
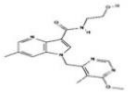
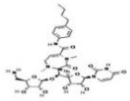
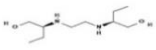
Table 2. Inhibitors Targeting Enzymes Involved in the Arabinogalactan Biosynthesis Pathway

Compound/ Inhibitor	Chemical class	Structure	Cell wall components inhibited	Ref
PBTZ169	Piperazinobenzothiazinone		DPrE1 Flavoprotein subunit of decaprenylphosphoryl- β -D-ribose-2-epimerase (DpRE1, Rv3790)	19
BTZ-O43	Benzothiazinone		DPrE1 Flavoprotein subunit of decaprenylphosphoryl- β -D-ribose-2-epimerase (DpRE1, Rv3790)	50
Benzothiazole Triazole	Benzothiazinone		DPrE1 Flavoprotein subunit of decaprenylphosphoryl- β -D-ribose-2-epimerase (DpRE1, Rv3790)	51
PyrBTZ01	Pyrrolebenzothiazinone		Inhibit arabinogalactan synthesis	52
TCA 1	Thiophene		Inhibit arabinogalactan synthesis	53
TBA7371	AzaindoleV		Arabinogalactan LAM	19
OPC-167832	3,4-Dihydrocarbostyril derivatives		Arabinogalactan LAM	5
CPZEN-45	Caprazene Nucleoside		WecA	5
Ethambutol and analogs	Ethylene diamino di-1-butanol		EmbA /EmbB Target synthesis and polymerization to affect lipid/cell wall synthesis.	8

compounds from different libraries that exhibited good pharmaceutical activity against MurA. These compounds included three from the ChemDiv library (D675-0217, D675-0102, and L291-0509) and three from the Asinex library (BDG 34016655, BDE 26717803, and BDE 25373574).[27] The identified peptidomimetic agents and derivatives have the potential to partially or completely block the substrate binding of MurA, thereby inhibiting downstream substrate synthesis for subsequent Mur enzymes. By targeting MurA, these compounds

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offer a potential strategy for the development of antimycobacterial agents to treat TB.

MurB and MurE Inhibitors. MurB is a crucial enzyme for bacterial cell survival as it reduces UDP-*N*-acetylenolpyruvoylglucosamine to UDPMurNAc. It is a potential drug target because it has no homologues in eukaryotic cells. Several inhibitors, including sulfadoxine, pyrimethamine, and piperazine derivatives (Figure 1), have been identified using structure-based drug discovery methods.[26] MurE, also known as UDP-*N*-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase, adds m-DAP to UDP-MurNAc-L-Ala-D-Glu. Glucosamine uridine analogues have been found to inhibit MurE in the cell wall synthesis pathway (Figure 1). Recent studies have identified potential inhibitors of MTB-MurB, including chloropicolinate amides,[26] as shown in Figure 1 and Table 1.

Alanine Racemase Inhibitor. The alanine racemase enzyme is responsible for catalyzing the conversion of L-alanine to D-alanine in the cytoplasm under the influence of pyridoxal phosphate. An analogue of D-alanine, known as D-cycloserine, is used as a second-line TB medication. D-Cycloserine prevents PG production by inhibiting the activity of D-alanine and alanine racemase (Table 1).[28]

L,D-Transpeptidase Inhibitor. Carbapenems are a class of antibiotics known to inhibit several enzymes involved in bacterial cell wall synthesis and maintenance, including D-transpeptidases, L,D-transpeptidase, and D-carboxypeptidases (Figure 1). LdtMt2, a target suppressed by carbapenems, has been investigated as a potential target for antituberculosis medications.[29] This study highlights the potential of capuramycin, tunicamycin, and muramycin D2 as novel inhibitors (Table 1) and offers promising avenues for further research and development.[26]

MurG and MurJ Inhibitor. MurG facilitates the transfer of GlcNAc from lipid-bound UDP-GlcNAc to MurNAc or MurNGlyc in lipid II (Figure 1). Certain lipoglycosidase peptide antibiotics such as ramoplanin and enduracidin bind to lipid components and inhibit the action of MurG[26] (Table 1). It is necessary to characterize the MurJ enzyme, and additional research will aid in the development of new MurJ inhibitors.[30] Various compounds, such as ramoplanin, teixobactin, malacidin, nisin, vancomycin, and the glycopeptide teicoplanin, bind to lipid II, hindering its elongation and affecting cell wall synthesis.[31]

■ ARABINOGALACTAN BIOSYNTHESIS AND POTENTIAL TARGETS

The polysaccharide backbone of AG is the main layer of the *Mycobacterium tuberculosis* cell wall (Figure 2). The AG backbone consists of galactose and arabinose sugar moieties. The backbone contains a linear chain of d-Galf with approximately 30 galactose units and three chains of Araf, each with approximately 30 arabinose residues.[39] The linker moiety connecting the PG and MA layers in the cell wall was synthesized using GlcNAc as the initial substrate, followed by intracellular modification by GlcNAc-1-P transferase (WecA) and rhamnosyl transferase (WbbL).[5] Linker synthesis begins in the cytoplasm and continues in the periplasm. Galactose residues are added to the PG linkage by galactofuranosyltransferases (GlfT1 and GlfT2), whereas arabinose residues are provided by decaprenylphosphoryl-D-arabinose (Araf).[40] Decaprenol-1-phosphoribose (DPR) production involves multiple enzymatic steps catalyzed by UbiA, PrsA, and DprE1/DprE2.[41] The addition of arabinofuranosyl residues to the AG backbone is catalyzed by arabinofuranosyl transferase (AftA) and Emb proteins (EmbA and EmbB).[5] AG was linked to the PG layer by Lcp1. Ethambutol, an antituberculosis drug, disrupts AG assembly by targeting arabinosyl transferase.

WecA Inhibitors. CPZEN-45, a novel semisynthetic compound developed from caprazamycin found in nature and created by *Streptomyces* species, is currently in the preclinical testing phase.[42] This nucleoside antibiotic suppresses MTB growth by targeting the *Mycobacterial* WecA enzyme (Figure 2). CPZEN-45 has been demonstrated to be effective against both replicating and nonreplicating bacteria in vitro. In mouse models, CPZEN-45 has demonstrated efficacy in managing both drug-sensitive and drug-resistant tuberculosis infections and exhibits synergistic effects when combined with other TB drugs. To enable inhalation delivery in humans, a coproduct in powder form was produced using spray-drying technology by combining capreomycin and CPZEN-45.[42]

WbbI, GlfT1/GlfT2, and UGM Inhibitors. Few studies have explored inhibitors of rhamnosyltransferase WbbL, an essential component of arabinogalactan assembly.[5] Preliminary research has suggested the potential of UDP-Galf or iminopentitol derivatives as inhibitors of GlfT1 or GlfT2 enzymes (Figure 2). The primary endeavor is to discover inhibitors of GlfT1, and GlfT2 has been focused on developing transition states or substrate mimics. These enzymes exhibit a strong preference for the UDP-D-Galp substrate over the UDP-D-Galf product, by over 90%.[39] Obtaining UDP-d-Galf, which is necessary for reverse reaction observations, poses a commercial challenge, making inhibitor screening tests difficult to establish.[39] The fluorinated exoglycal analogue UDP-Galf is a potent compound with a half-maximal inhibitory concentration (IC_{50}) of 180 μM as a GlfT2 inhibitor.[39] Thiazolidinone derivatives have also shown promise as GlfT2 inhibitors, analyzed through methods like "Molecular docking," "3D-QSAR," and "in silico ADMETox".[43] In the search for innovative approaches for the treatment of TB, it is important to consider the potential of multitargeting GlfT1, GlfT2, and UGM with drugs developed as "transition state analogs".[44]

DprE1 Inhibitors. Novel chemical entities (NCEs) have emerged as inhibitors of DprE1 that block its epimerase activity. These inhibitors can interact with DprE1 through covalent or noncovalent interactions. Noncovalent inhibitors include benzothiazinone (PyrBTZ01),[45] thiophene (TCA1),[46] quinoxaline,[47] dinitrobenzamide, thiadiazoles, azaindole, pyrazole pyridine, aminoquinolines, and piperidine amides. Covalent inhibitors include nitrobenzothiazoinone (BTZ), benzothiazole, triazole, and nitrobenzamide[48] (Figure 2). Notably, efforts to enhance the efficacy of BTZ have resulted in the discovery of noncovalent DprE1 inhibitors (Table 2), demonstrating potent activity in a mouse model of TB. Furthermore, benzimidazoles have been identified as DprE1 inhibitors through molecular modeling, suggesting their potential binding to the active site of the enzyme.[49]

■ MYCOLIC ACID BIOSYNTHESIS AND POTENTIAL TARGETS

The outer layer of the mAGP complex in *Mycobacterial* cell walls consists of MA, long-chain fatty acids with an AG layer, and "a-alkyl" (C24−C26) and "b-hydroxy" (C42−C62) chains derived from glycerol and trehalose. This hydrophobic lipid layer forms an impenetrable barrier that prevents the passage of small hydrophilic molecules, including antibiotics.[54]

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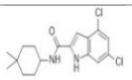
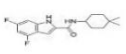
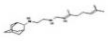
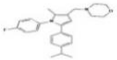
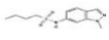
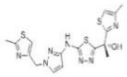
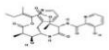
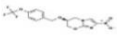
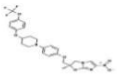
Table 3. Inhibitors Targeting the Mycolic Acid Biosynthesis Pathway's Enzymes

Compound /Inhibitor	Chemical class	Structure	Cell wall components inhibited	Ref
NITD 304	Indolcarboxamides		(MmpL3, Rv0206c)	64
NITD 349	Indolcarboxamides		MmpL3 Membrane transporter of trehalose monomycolate	64
SQ109	1,2 Ethylenediamine		MmpL3	65
BM212	1,5-Diarylpyrrole derivatives		MmpL3	63
BM635	1,5-Diarylpyrrole derivatives		MmpL3	63
AU1235	Adamantyl urea derivatives		MmpL3	5
Benzofurans	Amphetamine and phenylethylamine		Pks13	20
Cerulenin	Oxirane carboxylic acids and derivatives.		Pks13	20
GSK-724	Indazole sulfonamide		KasA/KasB β-ketoacyl-ACP synthase	66

Compound /Inhibitor	Chemical class	Structure	Cell wall components inhibited	Ref
TAC	Thiacetazone		HadABC Inhibit cyclopropanation of cell wall mycolic acid	19
Isoniazid	Isonicotinyl - hydrazide		inhA	19
ETO (Ethionamide)	Nicotinamide derivative (thioamide)		inhA Disrupt cell wall biosynthesis	19
GSK-693	Thiadiazole		inhA	65
Pyridomycin	Natural products		inhA	8
Pretomanid/ PA-824			Cell wall synthesis inhibition and causing respiratory poisoning through nitric oxide (NO) release.	67
TBA354	Nitroimidazoles		Inhibition of methoxy and keto-methoxy mycolic acid	2
Delamanid			The exact target is unknown	67

Mycolic acid biosynthesis involves two interconnected enzyme pathways, FAS I and FAS II[5] (Figure 3). FAS I produces short-chain (up to C24) fatty acyl coenzyme A (CoA), which is further elongated by FAS II to form the β-hydroxy (C42−C62) branch of mycolate. FabH catalyzes the conversion of FAS I byproduct "C14-CoA" to "C16-AcpM".[54] FabD transfers the malonyl group from malonyl-CoA to the acyl carrier protein (ACP) domain. The conversion of "C16-AcpM" to "C18-AcpM" involves several enzymatic steps mediated by MabA, HadAB/BC, inhA, KasA, and KasB.[55] MabA facilitates the reduction of β-ketoacyl-AcpM, thereby enabling fatty acid chain elongation. HadAB/BC and inhA are the dehydrated and saturated aliphatic chains, respectively. KasA and KasB, as β-ketoacyl synthases, condense "C18-AcpM" and "Malonyl-AcpM" to elongate the fatty acid chain. Each cycle of the FAS II system adds two carbons to the "AcpM" group, resulting in the production of a fully saturated acylated "β-hydroxy" (C42−C62) chain known as "C42−C62−AcpM".[56]

The FAS II byproduct undergoes several steps to produce mycolic acid. First, it is activated by the fatty acyl-AMP ligase "FabD" and then binds to the fatty acyl CoA (FAS I byproduct) in the presence of enzyme "PKs13".[57] The resulting compound was reduced by using Rv2509 to produce mycolic acid. The resistance-nodulation-division (RND) family of efflux pumps is used to transfer mycolic acids through the cell membrane. Mycolates produced in the cytoplasm are transformed into TMM, which is then effluxed by RND pumps called mycobacterial membrane protein enormous (MmpL).[20] After the efflux of TMM by MmpL, the mycolate on the translocated TMM is linked to AG by the mycolyl-transferase-antigen 85 complex, which completes the mAGP assembly. TMM is also converted to other membrane-unbound mycolates, such as trehalose dimycolates (TDM), by the antigen 85 complex.[56] MmpL3 is particularly important, because it is involved in the biosynthesis pathway of MA, the outermost layer of the cell wall (Figure 3). MmpL3 serves as a crucial target for developing tuberculosis medications, as it is responsible for transporting synthesized mycolates, including TMM, across the membrane.[58] A previous study by Ramesh et al. indicated that rimonabant, a CB1 receptor antagonist, shares a binding pocket similar to those of SQ109 and AU1235. The study showed that rimonabant had a 54 mM minimum inhibitory concentration (MIC) and could effectively limit the growth of MTB.[59] In a cell-based inhibition assay, the addition of the MmpL3 gene on a plasmid to the *M. smegmatis* strain partially reversed the inhibitory effect of rimonabant. These results provide evidence that MmpL3 is the primary target of rimonabant.[54] It has been reported that several novel chemical families including Indole-2-

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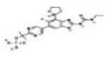
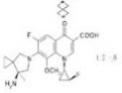
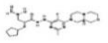
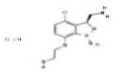
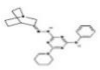
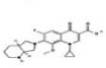
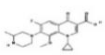
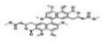
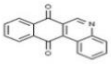
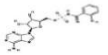
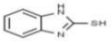
Table 4. Inhibitors Targeting Energy Metabolism and DNA Replication

Compound /Inhibitor	Chemical class	Structure	Cell wall components inhibited	Ref
SPR720	Aminobenzimid-azole		GyraseB Targeting DNA replication and Protein synthesis	88
Delpazoid (LCB01-0371)	Oxazolidinone		Targeting DNA replication and inhibits Protein synthesis	89
TBI-223	Oxazolidinone		Targeting DNA replication and protein synthesis	89
DC-159a	Fluoroquinolones		DNA replication	76
Linezolid	Oxazolidinones		Protein synthesis inhibition	90
AZD5847	Oxazolidinones		Protein synthesis inhibition	90
Actinonin	Type B peptide deformylase		Protein synthesis inhibition	91
LBM-415	Peptide deformylase		Protein synthesis inhibition	91
GSK-3036656 (GSK-656)	Oxaboroles		Leucyl tRNA synthetase rRNA	92
ATB107	Aminoglycosides		TrpC	32
Griselimycin	Fluoroquinolone		DnaN	88
Moxifloxacin	Fluoroquinolone		DNA gyrase and topoisomerases	32
Gatifloxacin	Fluoroquinolone		DNA gyrase and topoisomerases	94
Lysocin E	Non-ribosomal peptides		Targeting Vitamin k synthesis and Cell membrane synthesis	95
Benzyl Pyridinone	Pyridones		FabI Fatty acid synthesis	36
Viriditoxin	Secondary metabolites		FtsZ Cell division target	36
Dequalinum	Quinolone derivative		MsbC	96
Benzo[g]isoquinoline-5,10-diones	Isoquinolinediones		Mtr	96
Benzo[J]phenanthridine-7,12-diones	Phenanthridinediones		Mtr Bacillary redox homeostasis	77
Salicyl-AMS	Salicylamides		MbtA	97
Dihydroxybenzoate	Hydroxybenzoic acid derivatives		Mbtl	97
Benzimadazole-2-thione	Benzimidazoles		Mycobactin synthesis	32
N α -aroyl-N-aryl-phenylalanine-ides	Amides		RNA polymerase Targeting DNA replication and Protein synthesis	89
GSK-1322322	Peptide deformylase		Protein synthesis inhibition	
BRD4592	Azetidine derivative	NA	TrpAB Inhibiting Aminoacid biosynthesis	98
Fluorinated anthranilates	Aromatic carboxylic acid		Tryptophan synthesis	32

carboxamides, pyrroles, pyrazoles, benzimidazoles, spirocycles, piperidinol, benzothiazole amides, and adamantly urea, operate as MmpL3 inhibitor (Figure 3 and Table 3).[60]

NITD 304, NITD 349, and SQ109. Indolcarboxamides, such as NITD-304 and NITD-349, bind to MmpL3 and effectively inhibit both drug-susceptible and drug-resistant MTB (Table 3).[61] These compounds show promising efficacy against acute and chronic MTB infections in mouse models, with good safety profiles in preclinical tests.[5]

According to Sequella, tuberculosis medication has three potential mechanisms of action: inhibition of MmpL3, dissipation of proton motive force (PMF), and inhibition of menaquinone biosynthesis. It disrupts transport while increasing the TMM levels and decreasing the TDM levels. Clinical trials (NCT01358162, NCT0158636, and NCT01785186) have been conducted in the United States and South Africa. Phase 2b-3 studies in Russia showed promising results when SQ109

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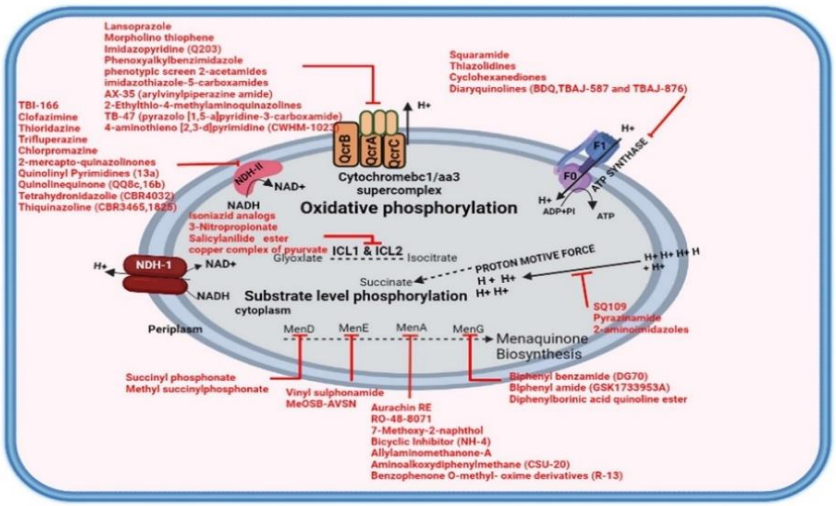

Figure 4. Oxidative phosphorylation and substrate-level phosphorylation are two interconnected mechanisms used by MTB to produce ATP. An arrow pointing in the direction of the target and inhibitor for various enzymes involved is shown in red text. It is unclear whether recent findings aimed at inhibiting menaquinone production will advance the development of anti-TB drugs.[107] Chemical inhibitors of MenA, MenB, MenG, MenD, and MenE are effective in limiting MTB growth. Further information about these compounds is presented in Table S.

was combined with other drugs, thereby demonstrating its safety and efficacy.[62]

BM212/BM635. The 1,3,5-trisubstituted pyrazole scaffold targets MmpL3, and two hits, "BM212" and "BM635," were obtained after optimizing the structure−activity relationship (SAR).[63] Both compounds demonstrated significant antitubercular activity and possessed desirable drug-like properties (Table 3). The effectiveness of TB mouse models has stimulated lead optimization in this series.[59]

KasA Inhibitors. Collaborative efforts between academic institutions and the pharmaceutical industry have led to the discovery of GSK 724, an indazole sulfonamide that exhibits antitubercular activity.[5] GSK 724 has shown promising results and is expected to undergo lead optimization soon. It acts as a *β*-ketoacyl ACP synthase (KasA) inhibitor and demonstrates synergistic effects when combined with INH (Table 3).[68]

InhA Inhibitors. Two of the currently used tuberculosis medications, INH and ETH, function by inhibiting enoyl-ACP reductase, also known as InhA.[69] InhA is involved in MA synthesis via the FAS II pathway. Prodrugs such as INH and ETH are activated to generate reactive oxygen species (ROS), which can impair InhA and interfere with MA chain elongation. The ORCHID consortium is creating chemical inhibitors based on thiadiazoles (GSK693) that actively block InhA (Table 3) and do not require cellular activation.[70] Other inhibitors of InhA include tetrahydropyrans, diaryl ethers such as triclosan (derivatives PT070 and PT119), methyl thiazoles, diazaborine, pyrrolidine carboxamides, and piperazine indole formamides, as reported previously (Figure 3).[71]

Pks13 Inhibitors. Indole II has been found to target the large polyketide synthase Pks13 (rv3800c), which is involved in MA biosynthesis, an essential component of the MTB cell wall.[72] Pks13, previously mentioned as a thiophene and benzofuran target, whereas benzofurans obstruct the active site of the C-terminal thioesterase domain (Table 3).[73] Thiophenes inhibit the loading of fatty acyl-AMP onto the N-terminal domain.[74]

OPC-67683 (Delamanid) and PA-824 (Pretomanid). OPC-67683 is a first-in-class bicyclic nitroimidazole drug developed by Otsuka Pharmaceutical for the treatment of MDR-TB.[75] It interferes with the formation of methoxy and keto-mycolic acids (Table 3) and has shown high potency against various forms of MTB in preclinical studies.[75] Delamanid has demonstrated safety and efficacy in human subjects with pulmonary MDR-TB, although the potential prolongation of the QTcF interval is a concern. A phase III clinical trial (NCT01424670) showed that the addition of delamanid to an optimized background regimen (OBR) did not provide any benefit.[67]

PA-824 is a nitroimidazole compound approved by the U.S. Food and Drug Administration (FDA) as part of a BPaL treatment regimen for XDR and/or MDR-TB.[76] It exhibits potent activity against both replicating and nonreplicating MTB strains. Although its specific target under aerobic conditions is unknown, it is believed to interfere with ketomycolate synthesis[77] (Table 3). During hypoxia, PA-824 is thought to induce respiratory toxicity through reactive nitrogen species (RNS) production.[78] The Nix-TB trial demonstrated a high recovery rate in patients with XDR-TB treated with BPaL, including PA-824.[79]

■ TARGETING DNA REPLICATION AND PROTEIN SYNTHESIS

Several drugs, such as fluoroquinolones, rifampin, streptomycin, kanamycin, and capreomycin, target DNA replication, protein

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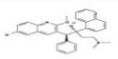
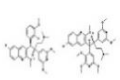
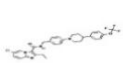
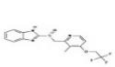
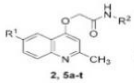
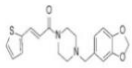
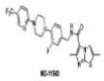
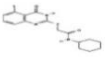
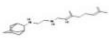
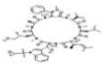
Table 5. Targeting Oxidative Phosphorylation and Proteolysis

Compound/ Inhibitor	Chemical class	Structure	Cell wall components inhibited	Ref
BDQ	Diaryquinolines		ATP synthase c-subunit (Rv1305) and other targets are unknown	36
TBAJ-587 and TBAJ-876	2nd generation Diary quinolines		ATP synthase c-subunit (Rv1305) and other targets are unknown	36
Q203 (Telacebac)	Imidazopyridine amide		Cytochrome b subunit of the cytochrome bc1 complex (Rv2196)	5
Lansoprazole	Prevacid		Cytochrome b subunit of the cytochrome bc1 complex (Rv2196)	108
2-(Quinolin-4-yloxy) acetamides	Acrylamides	 2, 5a-t	Cytochrome b subunit of the cytochrome bc1 complex (Rv2196)	5
Imidazole (2,1-b) thiazole-5-carboxamides	Thiazole containing heterocycles		Cytochrome b-subunit of the cytochrome bc1 complex (Rv2196)	109
AX-35	Arylvinylpiperazine amide		QcrB	110
ND-11543	Organic compound		QcrB	111
Clofazimine	Lamprene		NAD-II	112
Phenothiazines	Phenothiazines		NAD-II	112
CBR3465	Thiquinazoline		NAD-II	112
2-Mercapto-quinazolinones	Quinazolines		NAD-II	112
3-Nitropropionate	Carboxylic acid		ICL-I and ICL-II	5
Aurachin RE	Prenylated quinoline		MenA	32

Compound/ Inhibitor	Chemical class	Structure	Cell wall components inhibited	Ref
RO-48-8071	Oxazolidinones		MenA	113
Allylaminomethanone-A	Ketones		MenA	113
7-Methoxy-2-naphthol	Aromatic compound		MenA	114
DG-70 (GSK1733953A)	Biphenyl amide		MenG	107
SQ109	Ethylenediamine		Proton Motive Force	32
Cyclomarin A	Cyclic peptides		ClpC Inhibitor targeting proteolysis	115
Lassomycin	Cyclic tridecapeptide		ClpC Inhibitor targeting proteolysis	115
Ecumicin	Cyclic tridecapeptide		ClpC Inhibitor targeting proteolysis	115
Rufomycin	Cyclic Heptapeptides		ClpC Inhibitor targeting proteolysis	5
Squaramides	Vinylogous amides		ATP synthase c-subunit (Rv1305) and other targets are unknown	36
Tetrahydronidazole	Nitroimidazoles		NAD-II	112
Salicylanilide ester	Ester	NA	ICL-I and ICL-II	5
The copper complex of pyruvate isoniazid analogs	-		ICL-I and ICL-II	5
Bioisosteres sulphamate and Sulphamide	Vinyl sulphonamide MeOSB-AVSN		MenE	101
Vinyl sulphonamide group	Sulphonamide		MenE	101
Diphenylborinic acid quinoline ester	Boronic acid ester		MenG	107

synthesis, and transcription. These drugs effectively treat TB but represent only a subset of the available options.[80]

DNA Replication. Anti-TB drugs target crucial proteins involved in DNA replication and cellular division in MTB. One important target is *Mycobacterial* DNA gyrase, encoded by gyrA and gyrB, which unwinds DNA during replication.[81] Chemical inhibitors such as thiophene-based compounds have shown effectiveness but face challenges in clinical trials.[5] Considering the failure to obtain these thiophene-based compounds in clinical trials, fluoroquinolones prevent DNA gyrase activity,

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impair DNA replication, and cause permanent DNA breaks.[82] Moxifloxacin exhibited a sterilizing effect on both replicating and nonreplicating MTB strains. The lack of success in reducing the course of TB treatment in a phase III trial using the antibiotic moxifloxacin does not necessarily imply that DNA gyrase is not a desirable target for anti-TB drugs.[83] Griselymicin targets DnaN, a subunit of DNA polymerase III, and is effective against MTB replication (Table 4). It may also affect DNA repair pathways.[84]

DNA Transcription. *Mycobacterial.* RNA polymerase (RNAP) plays a crucial role in RNA transcription and elongation. RIF, an anti-TB medication, binds to the β-subunit of RNAP and inhibits RNA elongation.[85] RIF resistance arises from mutations in rpoB. Na-aroyl-*N*-aryl-phenylalaninamides (AAPs)[86] and pseudouridimycin also target RNAP (Table 4).[87] These compounds offer advantages such as anti-mycobacterial properties, lack of cross-resistance with RIF, synergistic effects when combined with RIF, and a lower likelihood of RIF-resistant strains emerging when used together with RIF. Prokaryotes and eukaryotes do not share RNAP, making it a unique target in terms of its specificity. As a result, RNAP continues to be underutilized, even though many commercially available drugs target it.[5]

■ TARGETING ENERGY METABOLISM

MTB relies on substrate levels and oxidative phosphorylation to generate ATP (Figure 4). This dual pathway reliance is driven by MTB's higher energy demands compared to those of other bacteria. Promising therapeutic candidates targeting both metabolic pathways are currently being developed for TB treatment.[99]

Substrate Level Oxidation. Under aerobic conditions, MTB utilizes glycolysis to generate acetyl-CoA from carbon sources, such as carbohydrates and fatty acids. Acetyl-CoA is then converted to citrate, isocitrate, and carbon dioxide in the tricarboxylic acid (TCA) cycle. The glyoxylate shunt converts isocitrate into glyoxylate and succinate (ICL I and ICL II), aiding carbon conservation.[100] Substrate-level glycolysis produces CO_2 and reduces NAD^+ to NADH. Reduced NADH drives oxidative phosphorylation by transferring electrons to the electron transport chain (ETC).[5]

Oxidative Phosphorylation Pathways. The OxPhos pathway is a promising target for drug development against MTB infection. FDA-approved medications such as BDQ, PA-824, and delamanid have validated the targeting of *Mycobacterial* ATP synthase and the OxPhos pathway for DR-TB treatment.[101] This was followed by the regulatory clearance of nitroimidazoles PA-824 and OPC-67683.[30] Telacebec (Q203) targets the cyt-bcc-aa3 complex, the main terminal oxidase of MTB (Table 5).[101]

The sensitivity of the OxPhos pathway to pharmaceutical inhibition and the conservation of this pathway make it an attractive therapeutic target. In many bacteria, substrate-level phosphorylation can supply sufficient energy for reproduction, whereas MTB relies on more energetically efficient OxPhos to keep growing.[99] This is likely due to *Mycobacteria* lacking an NADH-dependent lactate dehydrogenase, which would impede effective fermentation.[102] Inhibition of OxPhos can eliminate nonreplicating MTB and affect drug efflux pumps.[103] Although efflux pump activity has been shown to play a key role in MTB drug sensitivity, the indirect consequence of disrupting the OxPhos pathway may help overcome drug resistance mediated by efflux pumps.[101] Johnson et al. discovered and improved the inhibitor BRD-8000 for the efflux pump EfpA/RV2846c, thereby validating its potential as a therapeutic target.[104] Efforts have also focused on targets, such as tryptophan synthase, β-ketoacyl-ACP-synthase-I, biotin protein ligase, and leucyl-tRNA synthetase.[93,84]

■ TARGETING MENAQUINONE BIOSYNTHESIS

The remarkable significance of this pathway is that it is a compelling candidate for the development of anti-TB drugs (Figure 4).[95] Although efforts to inhibit menaquinone production have shown limited clinical significance, certain compounds such as alkylamino-methanone[105] and 7-methoxy-2-naphthol have been shown to bind to MenA and demonstrate activity against MTB (Table 5).[106] DG70, a biphenyl amide-based compound, shows promise in targeting MenG in the menaquinone biosynthesis pathway.[5]

F1F0 ATP Synthase Inhibitors. A key enzyme involved in ATP production is a major therapeutic target in MTB infections. BDQ, a diarylquinoline drug, has been approved by the FDA for the treatment of MDR-TB. It inhibits ATP synthesis by binding to specific subunits of ATP synthase and hinders the movement of the c-rotational subunit during ATP catalysis.[116] However, BDQ has limitations such as cardiotoxicity and the development of resistance. Mutations in AtpE have been associated with BDQ-resistant strains. These mutations are primarily found in the F0 region of ATP synthase.[116] To overcome these challenges, researchers are exploring the chemical space of diarylquinolines to discover next-generation analogues with improved efficacy and safety profiles.[117] Analogue compounds such as TBAJ-587 and TBAJ-876 have been investigated in preclinical studies, and squaramide-based compounds have shown promise in mouse models of TB infection.[118,119] Targeting energy metabolism through ATP synthase inhibition is an attractive approach for developing new anti-TB drugs.[120]

PMF Inhibitors. Proton motive force is an important component of bacterial energy metabolism, and its targeting has therapeutic implications. Compounds such as rotenone and CCCP act as inhibitors and protonophores, respectively, affecting proton transfer across the membrane.[101] Pyrazinoic acid, found in the first-line anti-TB drug regimen, has been shown to target PMF and decrease ATP levels.[121] TB drugs such as SQ109, BDQ, and CFZ also function as uncouplers with multiple targets, including PMF disruption.[101] Identifying compounds that specifically target *Mycobacterial* PMF disruption is crucial for the development of effective drugs to accelerate TB treatment.[101]

MTB utilizes the glyoxylate shunt pathway to conserve energy under anaerobic conditions, particularly within host macrophages and persister cells. Isocitrate lyase (ICL) is an essential enzyme in this pathway, and its inhibition leads to metabolic impairment of MTB.[122] Various small molecules, such as itaconate, itaconic anhydride, 3-bromopyruvate, oxalate, malate, and 3-nitropionate, have shown anti-mycobacterial activity by targeting the ICL.[5] Compounds such as salicylanilide derivatives, benzamide derivatives, phthalazinyl derivatives, and certain copper complexes have also exhibited ICL activities (Figure 4 and Table 5).[5]

Respiratory Poisoning. NO plays a crucial role in the innate immune response against intracellular infections such as TB.[101] Drugs such as PA-824 and DEL activate nitroreductase Ddn of MTB, leading to NO production.[123] The antibacterial activity of PA-824 under anaerobic conditions is attributed to the release of NO through the production of desnitroimidazole metabolites.[14] Under aerobic conditions, both PA-824 and DEL

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inhibited the synthesis of MA.[14] Transcriptomic studies have shown that the antibacterial activity of these drugs is dependent on NO poisoning in MTB.[14] Current efforts aim to discover inhibitors of the ETC components in MTB. Although the OxPhos pathway has been successfully targeted, potential inhibitors that resemble eukaryotic components or respond to specific conditions, such as Cyt-bd, have been overlooked.[101] Insights into ETC modulation under host conditions would aid the development of energy metabolism inhibitors.[124]

Targeting Proteostasis/Proteolysis. Targeting proteostasis and proteolysis is a novel therapeutic strategy for combating the survival of host cells.[125] The ClpP complex, consisting of ClpP1 and ClpP2, along with the chaperone proteins ClpC1 and ClpX, plays a crucial role in the proteolytic machinery of MTB.[126] Modifying these complexes can lead to inhibition, activation, or decoupling of their functions. Cyclic peptides derived from actinomycetes, such as lassomycin, cyclomarin, rufomycin, and ecumicin, have shown inhibitory effects on MTB growth by targeting these pathways.[127] Detailed information regarding these inhibitors is presented in Table 5.

■ CONCLUSION

The development of novel inhibitors targeting *Mycobacterium tuberculosis* is crucial for addressing the challenges posed by TB, including drug resistance. Future perspectives on TB treatment include targeting nonconventional sites of action, utilizing inhibitors that bind to different spatial sites of the same target, or inhibiting multiple biological pathways. Enzymes involved in cell wall formation have been identified as promising therapeutic targets, and comprehensive screening methods have led to the discovery of new drug candidates with potent anti-mycobacterial activity. This progress offers hope for combating drug resistance in TB. To effectively combat TB, the scientific community needs to integrate findings, address existing gaps, explore new treatment options, and maintain engagement with social and political entities.

■ ASSOCIATED CONTENT

Data Availability Statement

The data sets used in this study is available from the corresponding author on reasonable request

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Author Contributions

VK and BN: Conceptualization, analyzed the data, wrote the original draft, and reviewed the manuscript; AKV: analyzed the data, wrote the original draft, review, and editing; MC, SM, AKG, JMK, PP, BK, SR, SG: wrote the original draft, review, and editing. All authors have read and approved the final version of the manuscript. Consent and approval for publication was obtained from all authors.

Notes

Ethical approval was not required for this study. However, no human or animal studies have been associated with this study. The authors declare no competing financial interest.

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A review paper entitled “Revolutionizing Tuberculosis Treatment: Uncovering New Drugs and Breakthrough Inhibitors to Combat Drug-Resistant *Mycobacterium tuberculosis*”

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CERTIFICATE

This is to certify that Dr./Mr./Mrs./Ms. Ankit Verma
from Swami Rama Himalayan University, Dehradun
has participated / presented a paper (Oral /Poster) entitled Investigation of potential targets and their inhibitors in Mycobacterium tuberculosis
in the International Conference (BioSangam 2022) during March 10 - 12, 2022 at the Department of Biotechnology, MNNIT Allahabad, Prayagraj, India.

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This is to certify that Prof/Dr/Mr/Ms/ Ankit Verma Of Swami Rama Himalayan University presented poster titled Development Of Novel Inhibitors Targeting Murb Of Mycobacterium Cell Wall .In Biospectrum held from 5th to 7th November 2022

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