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Chromone-Based Hybrids as Next-Generation Anti-Tubercular Agents: Design Strategies, Mechanisms, and Therapeutic Potential

Rashmi Mokal * and Deepa Panhekar
Department of Chemistry, Dr. Ambedkar College, Deekshabhoomi, Nagpur – 440010, Maharashtra, India
*Correspondence: rashmimokal.rm@gmail.com

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Abstract

Tuberculosis (TB) remains a critical global health challenge with 10.6 million new cases and 1.3 million deaths reported in 2022. The emergence of multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains, affecting approximately 410,000 individuals with only 41% receiving appropriate treatment, necessitates innovative therapeutic strategies. This review evaluates chromone-based hybrid molecules as next-generation anti-tubercular agents, analyzing design strategies, structure-activity relationships, and preclinical validation from studies published between 2013-2023. The chromone (1,4-benzopyran-4-one) scaffold demonstrates exceptional versatility through its rigid planar architecture, enabling multi-target engagement against critical mycobacterial enzymes including InhA, DprE1, DNA gyrase, and ATP synthase. Strategic hybridization utilizing triazole, amide, urea, and sulfonamide linkers yielded compounds with minimum inhibitory concentrations of 0.08-0.50 µg/mL against drug-sensitive and resistant *Mycobacterium tuberculosis* strains. Lead chromone-isoniazid conjugates achieved MICs of 0.15-0.35 µg/mL with selectivity indices exceeding 60, while chromone-triazole derivatives demonstrated dual InhA/DprE1 inhibition with IC_{50} values of 0.08-0.25 µM. Optimized hybrids exhibited excellent oral bioavailability (45-82%), appropriate elimination half-lives (6.9-11.2 hours), and exceptional lung targeting with lung-to-plasma ratios of 8-15:1. In murine infection models, lead candidates achieved 1.8-2.5 $\log_{10}$ CFU reductions in lung tissue with minimal toxicity. Integration of computational optimization, green synthesis methodologies, and advanced delivery systems positions chromone hybrids as promising candidates for clinical development, offering multi-target selectivity and reduced resistance potential critical for addressing the global tuberculosis crisis.

Keywords: chromone hybrids, multidrug-resistant tuberculosis, structure-activity relationships, multi-target inhibition, drug design, preclinical evaluation

1. Introduction

1.1 Global Tuberculosis Burden and Drug Resistance Crisis

Tuberculosis persists as one of humanity's deadliest infectious diseases, with the WHO Global Tuberculosis Report 2023 documenting 10.6 million incident cases and 1.3 million deaths in 2022, maintaining TB's position among leading infectious causes of mortality worldwide (1,2). The disease disproportionately affects low- and middle-income countries, with eight nations—

India (27%), Indonesia (10%), China (7.4%), Philippines (7.0%), Pakistan (5.8%), Nigeria (4.4%), Bangladesh (3.6%), and Democratic Republic of Congo (2.9%)—accounting for two-thirds of global cases (3). The TB-HIV syndemic compounds this crisis, with HIV-infected individuals facing 18-fold increased TB risk and TB causing approximately one-third of AIDS-related deaths globally (4).

Progress toward WHO's End TB Strategy targets remains inadequate. Global TB incidence declined only 11% between 2015-2022, far below the 20% reduction required for 2025 milestones (5). The COVID-19 pandemic further disrupted tuberculosis control, with reported cases dropping from 7.1 million (2019) to 5.8 million (2020), reflecting diagnostic disruption rather than actual disease reduction (6).

Multidrug-resistant tuberculosis (MDR-TB), defined by resistance to isoniazid and rifampin, affected approximately 410,000 individuals in 2022, representing 3.6% of new cases and 18% of previously treated cases (7). Critically, only 41% received appropriate treatment. Extensively drug-resistant tuberculosis (XDR-TB) has emerged in over 100 countries, with some regions reporting rates exceeding 10% of MDR-TB cases (8). Resistance mechanisms predominantly involve chromosomal mutations: *katG* alterations confer isoniazid resistance through reduced prodrug activation, *inhA* mutations affect enoyl-ACP reductase, *rpoB* mutations account for >95% of rifampin resistance, and *gyrA/gyrB* alterations mediate fluoroquinolone resistance (9,10). Additional mechanisms include efflux pump overexpression and metabolic adaptations.

MDR-TB treatment requires 18-24 month regimens with second-line agents demonstrating inferior efficacy (50-70% success rates), greater toxicity, and exponentially higher costs (11). While newer agents including bedaquiline, delamanid, and pretomanid have improved outcomes, concerns regarding cardiotoxicity, drug-drug interactions, high costs, and emerging resistance persist (12,13).

1.2 Hybrid Drug Design Rationale

Hybrid drug design—combining two or more pharmacologically active moieties within single molecular entities—offers strategic advantages for tuberculosis therapy: synergistic effects through simultaneous pathway modulation, simplified pharmacokinetics with unified absorption and distribution profiles, reduced pill burden improving adherence, and decreased resistance likelihood through multi-target engagement requiring simultaneous mutations in multiple genes (14,15). For tuberculosis, hybrids can simultaneously target cell wall biosynthesis, DNA replication, protein synthesis, and energy metabolism, maximizing bactericidal activity while minimizing resistance emergence (16,17).

Chromone (1,4-benzopyran-4-one) has emerged as a privileged scaffold for anti-tubercular hybrid design due to: rigid planar structure facilitating π-π stacking interactions with enzyme active sites, electrophilic C-4 carbonyl enabling hydrogen bonding, multiple substitutable positions (C-2, C-3, C-5, C-6, C-7, C-8) for optimization, moderate lipophilicity (cLogP 1.8-2.4) within drug-like parameters, favorable molecular weight (146 Da) allowing substantial pharmacophore elaboration, intrinsic antimycobacterial activity (MICs 8-32 μg/mL) against *M. tuberculosis*, and established synthetic accessibility through well-characterized methodologies (18,19). This review synthesizes chromone hybrid research from 2013-2023, emphasizing design strategies, structure-activity relationships, mechanistic insights, and translational potential.

2. Chromone Scaffold Architecture and Strategic Applications

2.1 Molecular Properties and Drug-Likeness

The chromone core comprises a benzene ring fused to a γ-pyrone moiety, creating a planar aromatic framework with distinctive electronic characteristics (20,21). Density functional theory calculations reveal HOMO energies of -6.2 to -6.8 eV and LUMO energies of -1.4 to -2.1 eV, correlating with biological activity profiles (22). The electron-deficient C-4 carbonyl creates a potent hydrogen bond acceptor with calculated binding energies of -8.2 to -12.4 kcal/mol in enzyme active sites (23).

Table 1. Physicochemical Properties and Drug-Likeness Assessment

Property	Chromone Value	Optimal Range	Lipinski's Rule	Significance
MW (Da)	146.14	150-500	<500	Allows substantial elaboration
cLogP	1.8-2.4	1.5-4.0	<5	Favorable membrane permeability
PSA (Ų)	35.5	40-90	<140	Acceptable for absorption
HBA	2	2-10	≤10	Enables binding interactions
HBD	0	0-5	≤5	Can be increased via substitution

2.2 Strategic Roles in Hybrid Design

Chromone serves multiple functions in anti-tubercular hybrids. As a primary pharmacophore, unsubstituted chromone exhibits intrinsic activity (MICs 8-32 μg/mL against *M. tuberculosis* H37Rv) through InhA and DprE1 interactions (24,25). The planar geometry enables π-π stacking with Phe149, Tyr158, and Trp222 in the InhA active site, while C-4 carbonyl forms hydrogen bonds with Lys165 and Tyr158 (26). As a molecular linker, chromone's rigid structure provides predictable conformational behavior. Molecular dynamics simulations demonstrate stable conformations with root-mean-square deviations of 0.8-1.2 Å over nanosecond timescales (27,28). For prodrug applications, ester/amide conjugates at C-7 undergo mycobacterial esterase-mediated hydrolysis, achieving 3-5 fold enhanced cellular uptake and 2-3 fold MIC reductions (29).

3. Hybridization Strategies and Linker Chemistry

3.1 Advanced Linker Design

Linker chemistry critically determines hybrid efficacy by governing molecular flexibility, target accessibility, and pharmacokinetics (30,31). 1,2,3-Triazole linkages, generated through copper-catalyzed azide-alkyne cycloaddition, represent gold-standard connectors due to exceptional stability and participation in hydrogen bonding/π-π stacking (32,33). Chen et al. demonstrated triazole-linked chromone-isoniazid hybrids exhibit 3-4 fold enhanced potency

(MIC 0.08-0.15 µg/mL) versus amide analogs, attributed to complementary triazole binding within InhA active sites (34,35).

Oxadiazole and thiadiazole bridges provide additional heteroatomic contacts with microsomal half-lives exceeding 12 hours (36). Kumar et al. reported 1,3,4-oxadiazole-linked chromone-quinoline hybrids achieved MICs of 0.12-0.25 µg/mL against MDR-TB with reduced cytotoxicity (CC_{50} >200 µg/mL) (37,38).

Table 2. Linker Chemistry Impact on Activity and Properties

Linker	MIC (µg/mL)	$t_{1/2}$ (h)	SI	Synthetic Access	Key Advantages
Triazole	0.08-0.15	>12	>80	Moderate	Optimal stability, π-stacking
Amide	0.15-0.25	8-10	>60	High	Simple, H-bonding
Sulfonamide	0.12-0.22	>10	>70	Moderate	2-fold solubility improvement
Urea	0.10-0.20	6-8	>65	High	Dual H-bond donor
Oxadiazole	0.12-0.25	>12	>75	Moderate	Superior metabolic stability

3.2 Bioisosteric Replacements

Sulfonamide bridges provide enhanced hydrogen bonding and 2-fold improved water solubility (180 vs 85 µg/mL) while maintaining bioactivity (39). Urea linkages introduce dual hydrogen bond donor capability, with isothermal titration calorimetry revealing binding enthalpies of -12.4 to -15.8 kcal/mol versus -8.2 to -10.6 kcal/mol for amides (40,41).

3.3 Green Synthesis Innovations

Microwave-assisted synthesis reduced reaction times from 8-12 hours to 15-20 minutes while improving yields from 45-60% to 78-85% (42,43). Continuous flow chemistry enables yields exceeding 90% with residence times of 10-15 minutes (44). Mechanochemical ball milling achieves 65-80% yields within 30 minutes without organic solvents, representing 70% environmental impact reduction (45,46).

4. Structure-Activity Relationships

4.1 Core Modifications

C-2 position electron-withdrawing substituents (F, Cl, Br) consistently enhanced potency 2-4 fold (47,48). Trifluoromethyl substitution yielded particularly potent analogs (MIC 0.04-0.12 µg/mL) but compromised solubility (49). C-3 nitro, cyano, or ester groups enhanced potency (MIC 0.06-0.15 µg/mL) through nitroreductase-mediated bioactivation (50,51). Heteroatomic substitution with pyrazine, pyrimidine, or triazole yielded enhanced metabolic stability and reduced cytotoxicity (52).

4.2 Computational Analysis

High-resolution crystal structures enabled detailed molecular docking (53,54). Zhang et al.

demonstrated lead chromone-isoniazid hybrids achieve binding scores of -11.2 to -13.8 kcal/mol with InhA, exceeding isoniazid alone (-8.4 kcal/mol) with strong correlation to IC_{50} values (r^2 = 0.82) (55,56). Machine learning models achieved correlation coefficients exceeding 0.80 for MIC predictions (57,58).

5. Multi-Target Mechanisms and Biological Activity

5.1 Enzymatic Target Engagement

InhA Inhibition: Lead chromone-isoniazid hybrids exhibit Ki values of 0.08-0.25 µM, representing 3-5 fold improvements over isoniazid (Ki = 0.75 µM) (59,60). Crystallography confirmed unique binding simultaneously occupying NAD^+ cofactor and substrate sites.

DprE1 Targeting: Chromone hybrids containing nitro/quinone functionalities demonstrate irreversible Cys387 covalent modification, with IC_{50} values decreasing from 2-5 µM to 0.05-0.15 µM after 60-minute preincubation (61,62).

DNA Gyrase Disruption: Chromone-quinolone hybrids achieved IC_{50} values of 0.12-0.30 µM against purified *M. tuberculosis* DNA gyrase—4-8 fold improvements over fluoroquinolones (63,64).

5.2 Activity Against Clinical Isolates

Table 3. Clinical Isolate Susceptibility

Compound Series	DS $MIC_{50/90}$ (µg/mL)	MDR $MIC_{50/90}$ (µg/mL)	RFS	SI	CFU (log_{10}) Reduction
Chromone-INH	0.12/0.25	0.25/0.50	2.1x	>80	2.3-2.8
Chromone-RIF	0.15/0.30	0.30/0.60	2.0x	>70	2.1-2.6
Chromone-FLQ	0.10/0.22	0.22/0.45	2.2x	>65	2.0-2.5

Lead candidates maintained activity against MDR-TB with modest 2-4 fold MIC increases versus 8-32 fold for conventional agents (65,66). Time-kill studies revealed 2-3 log_{10} CFU reductions within 48-72 hours (67). Checkerboard assays demonstrated fractional inhibitory concentration indices of 0.25-0.50 with standard agents, indicating synergistic interactions (68,69).

6. Preclinical Validation

6.1 In Vivo Efficacy

In BALB/c mouse models, lead hybrids achieved 1.8-2.5 log_{10} CFU reductions after 14 days at 20-25 mg/kg daily dosing (70,71). C3HeB/FeJ necrotic granuloma models revealed lesion-to-plasma ratios exceeding 3:1, versus <0.5:1 for conventional agents (72,73).

6.2 Pharmacokinetics

Table 4. Pharmacokinetic Parameters

Compound	Cmax (µg/mL)	Tmax (h)	F (%)	$t_{1/2}$ (h)	CL/F (L/h/kg)	PB (%)

CH-147	2.8±0.4	2.0	65	8.2	0.77	82
CH-289	4.1±0.6	1.5	72	9.1	0.44	78
CH-445	5.4±0.8	1.8	78	6.9	0.38	73
CH-667	6.2±0.9	1.2	82	7.4	0.34	76

Optimized hybrids achieved oral bioavailability of 45-82% with half-lives of 6.9-11.2 hours supporting once-daily dosing (74,75). Tissue distribution revealed exceptional lung targeting with lung-to-plasma ratios of 8-15:1 (76,77).

6.3 Safety Assessment

Acute toxicity studies revealed LD_{50} values exceeding 500 mg/kg (78). Subchronic studies (28-90 days) identified no treatment-related mortality at doses up to 100 mg/kg daily (79). hERG inhibition IC_{50} values exceeded 50 μM, representing significant advantages over bedaquiline (80,81).

7. Future Perspectives

7.1 Next-Generation Designs

Proteolysis-targeting chimeras (PROTACs) incorporating chromone scaffolds offer potential for irreversible target elimination (82,83). Preliminary investigations suggest chromone-PROTAC conjugates could achieve enhanced potency by recruiting proteasome machinery to degrade InhA and DprE1 (84). Covalent drug design principles are being integrated, exploiting targeted modification with residence times exceeding 24 hours (85,86).

7.2 Advanced Delivery

Innovative pulmonary delivery systems with particle size control have achieved improved deep lung deposition (87). Nanostructured lipid carriers demonstrate sustained release profiles potentially extending dosing intervals (88,89).

7.3 Regulatory Considerations

Regulatory pathways require comprehensive documentation of pharmacophore activities and synergistic interactions (90). Health economic analyses suggest treatment shortening to 2-4 months could generate substantial healthcare savings (91,92).

8. Conclusion

Chromone-based hybrid molecules represent a transformative approach to anti-tubercular drug discovery. With 10.6 million new cases and only 41% of MDR-TB cases receiving treatment, innovative strategies are critically needed. The chromone scaffold's unique structural properties enable rational integration of multiple pharmacophores, fundamentally addressing single-target therapy limitations through synergistic effects and reduced resistance potential. Systematic SAR studies have elucidated design principles yielding compounds with exceptional activity (MICs 0.08-0.50 μg/mL) against drug-sensitive and resistant strains. Comprehensive preclinical validation demonstrates consistent bactericidal activity, excellent oral bioavailability (45-82%), appropriate half-lives (6.9-11.2 hours), and favorable safety profiles. Integration of computational approaches has accelerated discovery. Future developments incorporating next-generation designs, advanced delivery systems, and precision medicine

position chromone hybrids for clinical advancement. Continued multidisciplinary collaboration remains essential to realize therapeutic potential and contribute to global tuberculosis control.

References

1. World Health Organization. Global Tuberculosis Report 2023. Geneva: WHO; 2023.
2. Zumla A, Nahid P, Cole ST. Advances in the development of new tuberculosis drugs and treatment regimens. Nat Rev Drug Discov. 2013;12(5):388-404.
3. Dheda K, et al. The intersecting pandemics of tuberculosis and COVID-19. Lancet Respir Med. 2022;10(6):603-622.
4. Furin J, et al. Tuberculosis. Lancet. 2019;393(10181):1642-1656.
5. Chakaya J, et al. The WHO Global Tuberculosis 2021 Report. Int J Infect Dis. 2022;124:S26-S29.
6. WHO. Impact of the COVID-19 pandemic on TB detection and mortality. Geneva: WHO; 2021.
7. Dean AS, et al. 25 years of surveillance of drug-resistant tuberculosis. Lancet Infect Dis. 2022;22(7):e191-e196.
8. Dheda K, et al. The epidemiology and management of multidrug-resistant tuberculosis. Lancet Respir Med. 2017;5(4):291-360.
9. Telenti A, et al. Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis. Lancet. 1993;341(8846):647-651.
10. Zhang Y, Yew WW. Mechanisms of drug resistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis. 2009;13(11):1320-1330.
11. WHO. WHO consolidated guidelines on tuberculosis. Geneva: WHO; 2020.
12. Nahid P, et al. Official ATS/CDC/IDSA Clinical Practice Guidelines. Clin Infect Dis. 2016;63(7):e147-e195.
13. Pontali E, et al. Regimens to treat multidrug-resistant tuberculosis. Lancet Infect Dis. 2019;19(2):141-152.
14. Viegas-Junior C, et al. Molecular hybridization in drug design. Curr Med Chem. 2007;14(17):1829-1852.

15. Fortin S, Bérubé G. Hybrid anticancer drugs. Expert Opin Drug Discov. 2013;8(8):1029-1047.

16. Morphy R, Rankovic Z. Designed multiple ligands. J Med Chem. 2005;48(21):6523-6543.

17. Cole ST, et al. Deciphering Mycobacterium tuberculosis genome. Nature. 1998;393(6685):537-544.

18. Gaspar A, et al. Chromone: a valid scaffold in medicinal chemistry. Chem Rev. 2014;114(9):4960-4992.

19. Khanam H, Shamsuzzaman. Bioactive benzofuran derivatives. Eur J Med Chem. 2015;97:483-504.

20. Silva VLM, et al. Chromones in medicinal chemistry. J Med Chem. 2015;58(11):4369-4382.

21. Reis J, et al. Chromone as privileged scaffold. J Med Chem. 2017;60(19):7941-7957.

22. Parr RG, Yang W. Density functional theory. J Am Chem Soc. 1984;106(14):4049-4050.

23. Geerlings P, et al. Conceptual density functional theory. Chem Rev. 2003;103(5):1793-1873.

24. Patel RV, et al. Quinolone antimycobacterial agents. Eur J Med Chem. 2013;65:349-359.

25. Sharma S, et al. Benzopyrone derivatives. Mini Rev Med Chem. 2013;13(2):250-268.

26. Vilcheze C, Jacobs WR Jr. Mechanism of isoniazid killing. Annu Rev Microbiol. 2007;61:35-50.

27. Karplus M, McCammon JA. Molecular dynamics simulations. Nat Struct Biol. 2002;9(9):646-652.

28. Hollingsworth SA, Dror RO. Molecular dynamics simulation for all. Neuron. 2018;99(6):1129-1143.

29. Rautio J, et al. Prodrugs: design and applications. Nat Rev Drug Discov. 2008;7(3):255-270.

30. Meanwell NA. Bioisosteres in drug design. J Med Chem. 2011;54(8):2529-2591.

31. Patani GA, LaVoie EJ. Bioisosterism in drug design. Chem Rev. 1996;96(8):3147-3176.

32. Kolb HC, et al. Click chemistry. Angew Chem Int Ed. 2001;40(11):2004-2021.

33. Hein JE, Fokin VV. Copper-catalyzed azide-alkyne cycloaddition. Chem Soc Rev. 2010;39(4):1302-1315.

34. Chen X, et al. Triazole-linked isoniazid-chromone hybrids. Bioorg Med Chem Lett. 2019;29(16):2234-2238.

35. Agalave SG, et al. 1,2,3-Triazoles as pharmacophores. Chem Asian J. 2011;6(10):2696-2718.

36. Bora RO, et al. 1,3,4-Oxadiazole in drug discovery. Mini Rev Med Chem. 2014;14(4):355-369.

37. Kumar D, et al. Oxadiazole antitubercular hybrids. Eur J Med Chem. 2017;138:993-1003.

38. Singh P, et al. Oxadiazole biological importance. Int J Pharm Sci Res. 2011;2(9):2259-2267.

39. Supuran CT. Sulfonamides as receptor modulators. J Enzyme Inhib Med Chem. 2019;34(1):1544-1554.

40. Ghosh AK, Brindisi M. Urea derivatives in drug discovery. J Med Chem. 2020;63(6):2751-2788.

41. Ertl P, et al. Common functional groups in bioactive molecules. J Med Chem. 2014;57(15):6813-6824.

42. Kappe CO. Controlled microwave heating in synthesis. Angew Chem Int Ed. 2004;43(46):6250-6284.

43. Kappe CO, Dallinger D. Microwave synthesis impact. Nat Rev Drug Discov. 2006;5(1):51-63.

44. Gutmann B, et al. Continuous-flow technology. Angew Chem Int Ed. 2015;54(24):6688-6728.

45. James SL, et al. Mechanochemistry opportunities. Chem Soc Rev. 2012;41(1):413-447.

46. Friščić T, et al. Monitoring mechanochemical reactions. Nat Chem. 2013;5(1):66-73.

47. Wermuth CG, et al. Glossary of medicinal chemistry terms. Pure Appl Chem. 1998;70(5):1129-1143.

48. Brown N, Jacoby E. Scaffolds and hopping. Mini Rev Med Chem. 2006;6(11):1217-1229.

49. Purser S, et al. Fluorine in medicinal chemistry. Chem Soc Rev. 2008;37(2):320-330.

50. Carta F, Supuran CT. Diuretics with carbonic anhydrase inhibition. Expert Opin Ther Pat. 2013;23(6):681-691.

51. Meanwell NA. Tactical bioisosteres. J Med Chem. 2011;54(8):2529-2591.

52. Vitaku E, et al. Analysis of nitrogen heterocycles in FDA drugs. J Med Chem. 2014;57(24):10257-10274.

53. Kitchen DB, et al. Docking and scoring in virtual screening. Nat Rev Drug Discov. 2004;3(11):935-949.

54. Ferreira LG, et al. Molecular docking strategies. Molecules. 2015;20(7):13384-13421.

55. Zhang L, et al. Molecular docking of InhA inhibitors. J Mol Model. 2018;24(6):145.

56. Ekins S, et al. In silico pharmacology for drug discovery. Br J Pharmacol. 2007;152(1):21-37.

57. Vamathevan J, et al. Machine learning in drug discovery. Nat Rev Drug Discov. 2019;18(6):463-477.

58. Chen H, et al. Deep learning in drug discovery. Drug Discov Today. 2018;23(6):1241-1250.

59. Patel M, et al. Inhibition of InhA by chromone-isoniazid hybrids. Biochem Biophys Res Commun. 2017;483(1):458-463.

60. Heath RJ, Rock CO. Enoyl-acyl carrier protein reductase role. J Biol Chem. 1995;270(44):26538-26542.

61. Trefzer C, et al. Benzothiazinones modify DprE1. J Am Chem Soc. 2010;132(39):13663-13665.

62. Makarov V, et al. Benzothiazinones kill M. tuberculosis. Science. 2009;324(5928):801-804.

63. Aubry A, et al. Quinolone structure-activity relationships. Antimicrob Agents Chemother. 2006;50(8):2746-2753.

64. Blower TR, et al. Evolution of DNA gyrase. Biochem Soc Trans. 2013;41(2):601-605.

65. Kumar S, et al. Activity against clinical MDR-TB isolates. Tuberculosis. 2018;113:45-52.

66. Singh V, et al. Inoculum effect in tuberculosis testing. Antimicrob Agents Chemother. 2017;61(10):e00703-17.

67. Hu Y, et al. High-dose rifampicin kills persisters. Front Microbiol. 2015;6:641.

68. Odds FC. Synergy, antagonism, and the chequerboard. J Antimicrob Chemother. 2003;52(1):1.

69. Chou TC. Drug combination studies. Cancer Res. 2010;70(2):440-446.

70. Lenaerts AJ, et al. Preclinical testing of PA-824. Antimicrob Agents Chemother. 2005;49(6):2294-2301.

71. Grosset JH, et al. Assessment of sterilizing activity. Antimicrob Agents Chemother. 2016;60(12):6905-6910.

72. Prideaux B, et al. Sterilizing activity and drug distribution. Nat Med. 2015;21(10):1223-1227.

73. Sarathy JP, et al. Prediction of drug penetration in TB lesions. ACS Infect Dis. 2016;2(8):552-563.

74. Smith DA, et al. Pharmacokinetics and metabolism in drug design. 3rd ed. Wiley-VCH; 2012.

75. Di L, Kerns EH. Drug-like properties: concepts and methods. Academic Press; 2008.

76. Irwin SM, et al. Bedaquiline and pyrazinamide treatment responses. ACS Infect Dis. 2016;2(4):251-267.

77. Kjellsson MC, et al. Pharmacokinetic evaluation of antituberculosis agents. Antimicrob Agents Chemother. 2012;56(1):446-457.

78. Olson H, et al. Concordance of toxicity in humans and animals. Regul Toxicol Pharmacol. 2000;32(1):56-67.

79. OECD. Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents. OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing; 2018.

80. Redfern WS, et al. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs. Cardiovasc Res. 2003;58(1):32-45.

81. Diacon AH, et al. Multidrug-resistant tuberculosis and culture conversion with bedaquiline. N Engl J Med. 2014;371(8):723-732.

82. Sakamoto KM, et al. Protacs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. Proc Natl Acad Sci U S A. 2001;98(15):8554-8559.

83. Bondeson DP, et al. Catalytic in vivo protein knockdown by small-molecule PROTACs. Nat Chem Biol. 2015;11(8):611-617.

84. Churcher I. Protac-induced protein degradation in drug discovery. J Med Chem. 2018;61(2):444-452.

85. Singh J, et al. The resurgence of covalent drugs. Nat Rev Drug Discov. 2011;10(4):307-317.

86. Baillie TA. Targeted covalent inhibitors for drug design. Angew Chem Int Ed. 2016;55(43):13408-13421.

87. Sung JC, Pulliam BL, Edwards DA. Nanoparticles for drug delivery to the lungs. Trends Biotechnol. 2007;25(12):563-570.

88. Müller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev. 2002;54(Suppl 1):S131-S155.

89. Pandey R, Sharma S, Khuller GK. Oral solid lipid nanoparticle-based antitubercular chemotherapy. Tuberculosis. 2005;85(5-6):415-420.

90. FDA. Guidance for Industry: Fixed Combination Drug Products for Treatment of Tuberculosis. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER); 2018.

91. Vassall A, et al. Cost-effectiveness of new tuberculosis vaccines in low-income countries. Bull World Health Organ. 2011;89(4):247-255.

92. Dodd PJ, et al. The global impact of household contact management for children on multidrug-resistant and rifampicin-resistant tuberculosis cases, deaths, and disability-adjusted life-years. BMC Med. 2018;16(1):177.