REVIEW ARTICLE

Unlocking the Potential of Pyrazines: Structure–Activity Relationship Driven Studies against *Mycobacterium tuberculosis*

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Abstract

Tuberculosis (TB) is one of the diseases that has affected a wide population in the world, and due to the constant development of resistance, it has necessitated the development of potent drugs that can show anti-tubercular activity on the resistant strains as well. Among nitrogen-containing heterocycles, pyrazine has attracted significant attention in medicinal chemistry due to its chemical simplicity and adaptability in drug design. The current review provides the summary of pharmacological aspects of pyrazine derivatives as an anti-tubercular agent, focusing on examining the structure–activity relationship (SAR) trends, minimum inhibitory concentration (MIC) values as well as the key modifications, such as integrating the electron-withdrawing groups, heterocyclic linkers, and lipophilic side chains, that have led to the discovery of potent anti-tubercular drugs. The SAR studies suggest that the addition of electron-withdrawing groups and heterocyclic linkers has resulted in compounds with improved efficacy compared to the pyrazinamide. The evidence also provides significant insights into the therapeutic potential of pyrazine derivatives as scaffolds for next-generation anti-TB drugs.

Key words: Anti-tubercular, drug resistance, pyrazine, structure-activity relationship

INTRODUCTION

Pyrazine is a simple six-membered nitrogen-containing heteroaromatic scaffold that is used as a versatile pharmacophore owing to its structural simplicity and adaptability as a pharmacophoric unit.[1,2] The presence of two ring nitrogens confers distinct electronic characteristics that facilitate functional modification while maintaining favorable physicochemical properties.[3,4] The clinical relevance of the pyrazine scaffold is best illustrated by pyrazinamide (PZA), a cornerstone agent in standard tuberculosis (TB) therapy.[5,6] PZA is a prodrug that is metabolized into pyrazinoic acid (POA), which disrupts the mycobacterial membrane potential and fatty acid synthesis.[7] The pyrazine nucleus has been found to prominently contribute to several clinically important drugs, due to their promising pharmacological activity, stability, and binding characteristics.[8] These properties make pyrazine an ideal scaffold for drug design. For the pyrazine nucleus-containing compounds, the studies have identified some most important pharmacological targets, such as fatty acid synthase I (FAS-I), dihydrofolate reductase, and other metabolic enzymes, as well as mycobacterial biofilm formation involved in biosynthesis of cell wall, have been identified as potential therapeutic targets for pyrazine-based compounds.[9-13] However, its therapeutic utility is hindered by unaccountable resistance development, limited activity under neutral pH, and adverse effects, which have necessitated the innovation of novel analogues with enhanced efficacy. The incorporation of various substituents at different positions of the pyrazine ring has led to the identification of compounds

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with promising activity against *Mycobacterium tuberculosis*, including the resistant strains.[14-17] The different mechanism of action of pyrazine-containing compounds involve multiple targets that are depicted in Figure 1, thereby enhancing their broad-spectrum activity and minimizing the probability of resistance emergence.[7,18] The SAR studies have provided critical insights into the molecular features that are required for the optimal anti-tubercular activity within the pyrazine series.[19] Newer methods of drug designing, such as *in silico* molecular docking studies and quantitative SQR modeling, have further accelerated the rational design of novel pyrazine derivatives.[20,21] There are so many pyrazine-containing drugs available in the market, which is shown in Figure 2.

Morinamide, one of the derivative of PZA, as shown in Figure 3, was investigated in the 1980s, is a pyrazine-2-carboxamide derivative and structural analogue of PZA, acting as a prodrug that is hydrolyzed into POA.[22,23] POA disrupts *M. tuberculosis* by impairing membrane potential and energy production, particularly in acidic intracellular environments.[24,25] The morpholine moiety present in morinamide helps in increasing the lipophilicity, which increases the cell wall penetration and oral bioavailability.[26] However, it was not approved by the World Health Organization and is absent from current first- or second-line TB regimens. It is occasionally used in local or experimental protocols, but the global regulatory approval is lacking due to limited clinical data and comparative efficacy.[5,27]

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The chemical modification of the pyrazine-2-carboxamide nucleus has been explored as a strategy to produce novel and potent anti-tubercular agents. Introduction of electron-donating groups like -CH_3 or -NH_2, and electron-withdrawing groups such as -X (Cl, Br, F) or NO_2 or -CF3 has been found to significantly affect the biological activities by affecting their properties such as lipophilicity, electronic character, and membrane permeability that are essential for the proper functioning of bacteria.[28-32] In addition to functional group modifications, incorporation of heterocyclic moieties such as triazoles, oxadiazoles, thiophenes, and piperazines has contributed to improved antimycobacterial activity in many reported series.[33,34] Such scaffolds may offer better pharmacokinetic and pharmacodynamic profiles, in part due to their metabolic stability and favorable interaction with biological targets.[35,36] This review focuses on the evaluation of a series of pyrazine-based derivatives where a range of heterocyclic rings with different electron-donating and electron-withdrawing groups are substituted, which will provide valuable relationships for further lead optimization.[37-39] This review examines the several reports during the years 2010 to 2025 using search engines such as PubMed, Google Scholar, and EBSCO. This analysis aims to provide insights into the future directions for pyrazine-based TB drug discovery and development.

MECHANISM OF ACTION OF PZA

PZA, exerts its anti-tubercular mycobacterial activity through different mechanisms involving both confirmed and proposed targets, as represented in Figure 4.[40] Rather than acting directly, PZA requires intracellular activation and is converted to its active form, POA by the enzyme called pyrazinamidase.[41] POA accumulates inside the bacterial cell, particularly under acidic conditions, leading to acidification of the cytoplasm that results in the disruption of membrane energetics and transport functions, leading to cell death.[18] Beyond this general membrane disruption, POA and certain analogues, such as 5-chloro PZA, have been shown to competitively inhibit FAS-I at the NADPH-binding site, disrupting the synthesis of mycolic acids, which is essential for cell-wall integrity.[42,43] Another confirmed mechanism

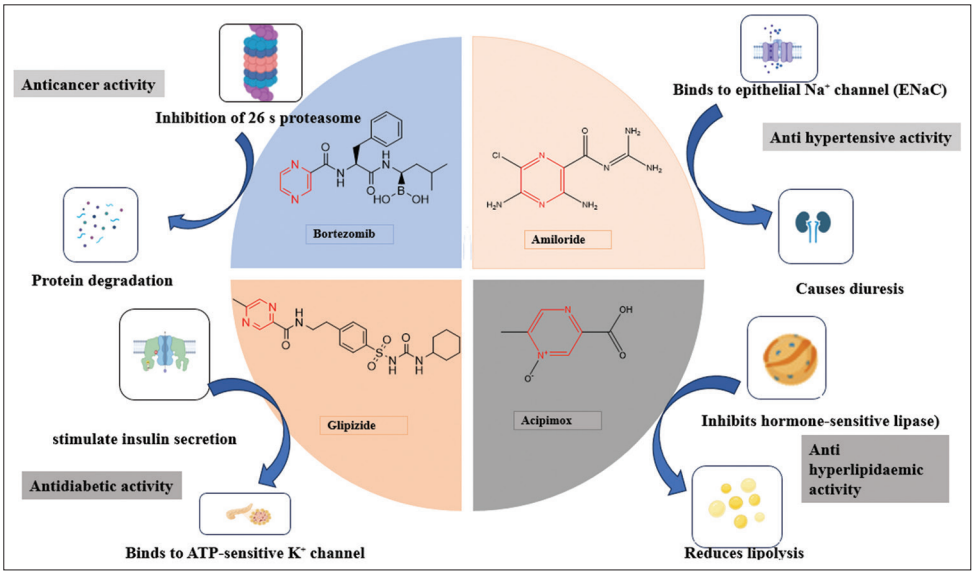

Figure 1: Mechanistic insights into marketed pyrazine-based drugs

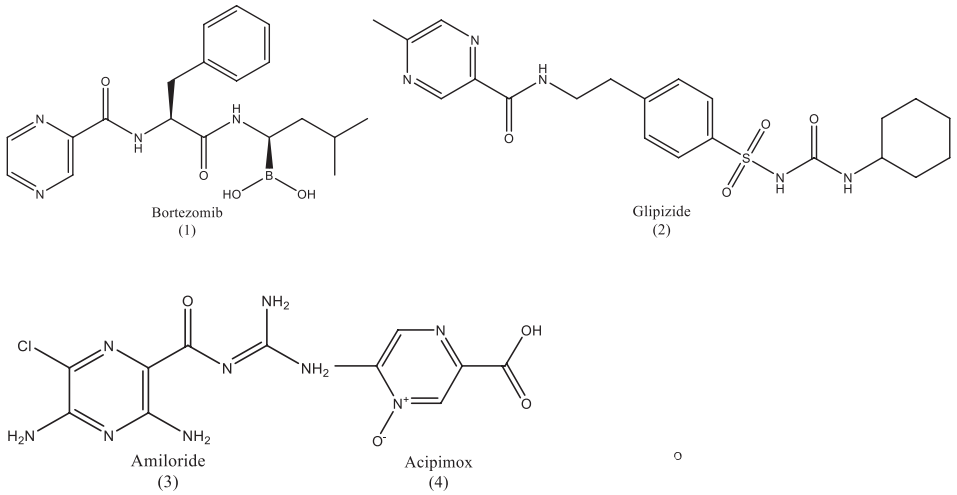

Figure 2: Pyrazine-containing marketed drugs

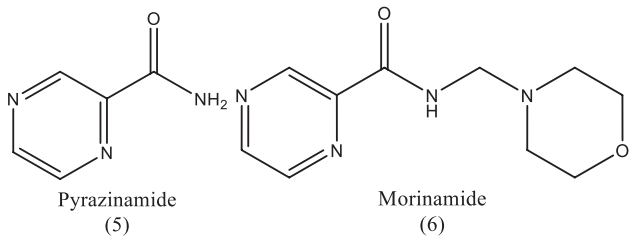

Figure 3: Structural analogs of pyrazinamide

involves the inhibition of trans-translation: POA binds to ribosomal protein S1, preventing transfer-messenger RNA interaction and thereby blocking this essential ribosome rescue pathway.[7] Additional proposed mechanisms include inhibition of NAD biosynthesis, disruption of coenzyme A, and suppression of (p)ppGpp synthesis by targeting guanosine pentaphosphate synthase, inhibition of enoyl-ACP reductase (InhA).[44-46]

PYRAZINE-BASED ANALOGUES ASPOTENT ANTI-TB AGENTS

Ortho-substituted pyrazine derivatives

Sriram *et al*. developed and synthesized a series of PZA mannich base-incorporated piperazine-substituted derivatives and screened for their anti-tubercular activity against H37Rv. Compound 7, containing a fluoroquinolone–urea–piperidine hybrid, showed notable efficacy, with an MIC of 0.39 (against MTB) and 0.2 µg/mL (against multidrug-resistant TB [MDR-TB]), respectively, compared to the standard drug PZA with the MIC value of 12.5 µg/mL (MTB) and >25 µg/mL (MDR-TB).[32,47] Hassan *et al.* designed and synthesized pyrazine-incorporated carboxamide, pyrazole carbothioamide, and thiophene-based novel derivatives and screened the compounds for their anti-tubercular efficacy. The compounds 8(a-d) contain an electron-withdrawing group such as bromo, chloro, and an electron-releasing group methyl group. The compound 8b, containing the electron-withdrawing bromogroup, exhibited great efficacy with the MIC value of 0.78 µg/mL. Compound 9, containing the heterocyclic ring thiophene, also showed good anti-tubercular activity with the MIC value of 6.25 µg/mL. Compound 10, containing a pyrazole carbothioamide group, also showed enhanced anti-tubercular activity with the MIC value of 3.12 µg/mL. All these compounds showed higher or equal anti-tubercular activity, with the reference drug PZA having an MIC value of 6.25 µg/mL.[31,48,49] Das synthesized pyrazine-1,3,4-oxadiazole-based derivative and incorporated substituents such as NH, NH_2, triazole, and tetrazole groups. Compound 11, which contains a 1,3,4-oxadiazole hydrazino group, showed the highest activity with the MIC value of 6.25 µg/mL compared to the standard drug rifampicin with the MIC value of 0.25 µg/mL and isoniazid with the MIC value of 0.20 µg/mL.[50] Zhou *et al.* synthesized through rational drug design pyrazine-carboxamide derivatives incorporating different heterocyclic rings with the alkyl linker. Compound 12a – containing a piperazine ring, compound 12b – containing a morpholine ring, and compound12c – containing a thiomorpholine ring (containing oxygen that is likely to increase the hydrogen-bond acceptor capacity) showed higher activity with the MIC value of 12.2, 8, and 10.2 µg/mL, respectively, which was higher than that of the standard PZA that has the MIC value of 12.5 µg/mL.[51] Zulquramin *et al.* rationally designed and synthesized a pyrazine carboxamide derivative and assessed the compounds for their anti-tubercular activity. Compound 13a, containing an electron-withdrawing group, chlorophenyl group and13b containing the cycloheptyl substituent, showed enhanced activity with MICs of each of lesser than or equal to 6.25 µg/mL, which was 16 times potent than that of the standard drug, PZA, which has an MIC value of 100 µg/mL.[52] Suryawanshi *et al.* designed and synthesized a series of pyrazine oxadiazole derivatives and screened them for their anti-tubercular activity. Compounds 14a and 14b

both have an electro-withdrawing group, Br, and F, while 14c contains an electron-donating group, OCH_3, and an electron-withdrawing group, Br, with MIC values of 3.13 µg/mL each, compared to the standard drug, isoniazid, with an MIC value of 0.4 µg/mL.[53] Naik*et al.* synthesised pyrazine incorporating a triazole heterocyclic ring by drug design and evaluated the compounds for their anti-tubercular activity. Compound 15, containing the electron-withdrawing group trifluoromethyl, showed the maximum potency among the series and was found to have the same MIC value as that of PZA, with an MIC value of 3.12 µg/mL.[34,54] Shivakumar *et al*. synthesized drug design pyrazine carbohydrazide derivatives and evaluated their anti-tubercular activity. Compounds 16a and 16b contain electron-withdrawing groups, bromo and nitro groups, whereas compound 17 with the nitrophenyl group showed greater potency than the standard drug PZA (MIC value of 3.12µg/mL), with each MIC value of 1.56 µg/mL.[55]

The compounds' chemical structures are shown in Figure 5, and the MIC value with the structural feature is shown in Table 1.

Ortho-meta-substituted pyrazine derivatives

Dolezal *et al.* synthesized pyrazine carboxamides substituted derivatives and tested for their anti-mycobacterial activity. Compound 18a, containing trifluoromethyl, and compound 18b, containing trifluoromethyl and a tert-butyl group as a substituent, showed maximum anti-tubercular activity with a MIC value of 6.25 and 3.13 µg/mL, respectively.[56] Dolezal *et al.* synthesized pyrazine carboxamide-aryl derivatives and evaluated anti-tubercular activity. Compound 19a, containing iodide and a methyl group, and compound 19b, containing additional tert-butyl and an electron-withdrawing

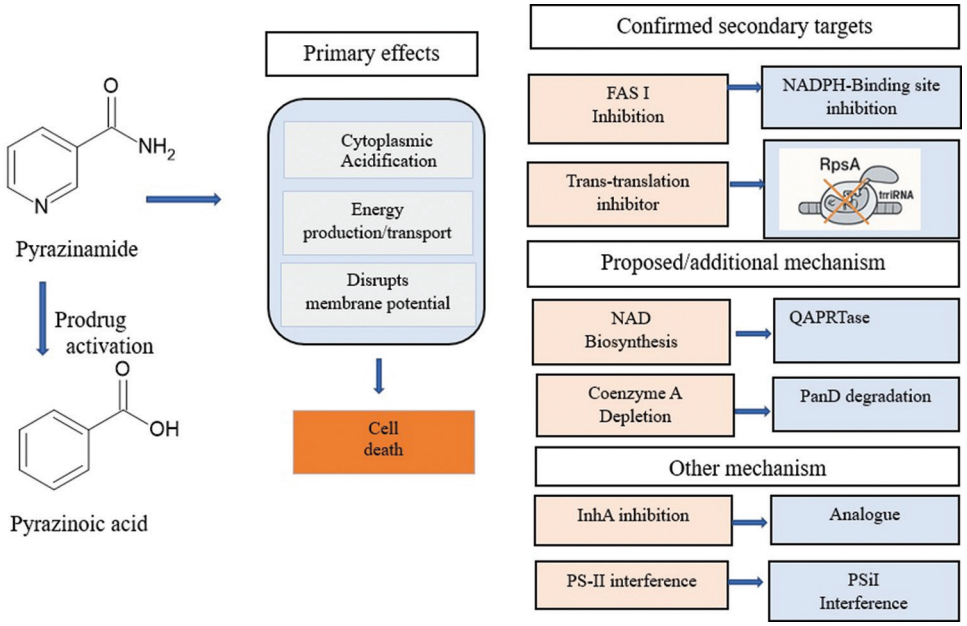

Figure 4: Different anti-tuberculosis mechanistic pathways for the pyrazine ring-containing reported drugs

Table 1: MIC values of pyrazine-containing derivatives

Compound code	Structural features	MIC value	Reference
1	Quinoline and pyrazine	0.39	Sriram *et al.* (2006)
2	Pyrazole and pyrazine	0.78	Hassan *et al.* (2020)
3	Thiophene and pyrazine	6.25	
4	Pyrazoline and pyrazine	3.12	
5	1,3,4-oxadiazole and pyrazine	6.25	Das (2015)
6	Morpholine, thiomorpholine, piperazine, and pyrazine	12.2, 8 and 10.2	Zhou *et al.* (2017)
7	Cycloheptane and pyrazine	6.25	Zulquramin *et al.* (2023)
8	1,3,4-oxadiazole and pyrazine	3.13	Suryawanshi *et al.* (2025)
9	Triazole and pyrazine	3.12	Naik *et al.* (2024)
10	1,3,4-oxadiazole and pyrazine	1.56	Shivakumar *et al.* (2025)
11	7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine and pyrazine	1.56	

MIC: Minimum inhibitory concentration

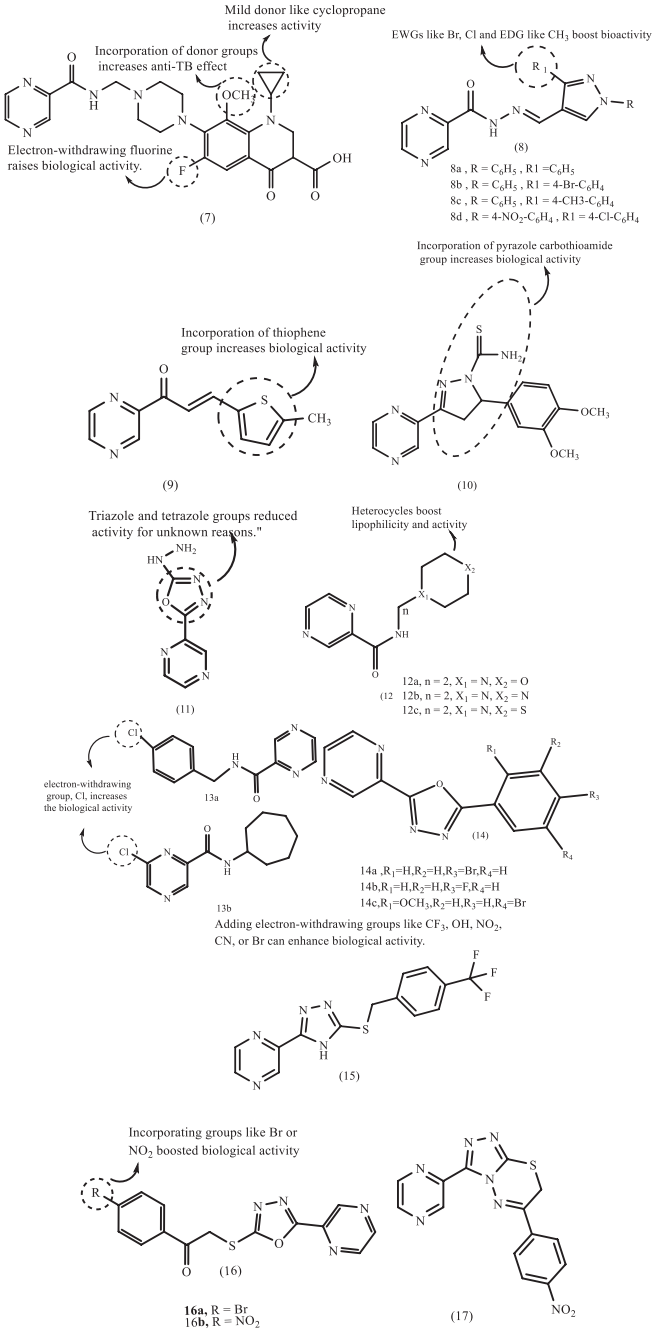

Figure 5: Compounds containing ortho-substituted pyrazine

group, chlorine, showed four times higher potency with MIC values of <2 µg/mL and 4 µg/mL, respectively, than that of the standard drug PZA (MIC value 8 µg/mL).[57,58] Zitko *et al.* synthesized pyrazine carboxamides and evaluated the compounds for their anti-tubercular activity. Compound 20a contains electron electron-withdrawing group, chloride, and an electron-donating group-OH, whereas compound 20b, containing an electron-withdrawing group -COOH and an electron-donating group -OH, showed maximum potency with the MIC value of 1.56 and 3.13 µg/mL, respectively, whereas the standard drug PZA has a MIC value of 6.25–12.5 µg/mL.[59] Servusová *et al.* synthesized pyrazine carboxamide derivatives for their anti-tubercular activity. Compound 21a, containing the electron-withdrawing group chloride, and compound 21b containing the electron-donating group methyl, showed maximum potency with the MIC value of 6.25 and 3.13 µg/mL, respectively, against *Mycobacterium kansasii*, to which the standard compound PZA isunsusceptible.[60] Zitko *et al.* designed and synthesized pyrazine carboxamide derivatives and their compounds for their anti-tubercular activity. Compound 22a, containing the electron-withdrawing group fluoro group, compound 22b and 22c containing the electron-withdrawing group chloro, and compound 22d containing the electron-withdrawing group iodo showed higher potency of each MIC value of 3.13 µg/mL compared to the reference drug PZA with the MIC value of 12.5 µg/mL.[61] Jandourek *et al.* synthesized pyrazine carbonitrile derivatives and studied the compounds for their anti-tubercular activity. Compounds 23a and 23b, containing an electron-withdrawing group, chloride, and 23c, containing an electron-withdrawing group, trifluoromethyl group, showed maximum potency with the MIC value of 6.25 µg/Ml against H37Rv TB compared to the standard drug PZA with the MIC value of 12.5 µg/mL.[62] Semelkova *et al.* designed and synthesized pyrazine carboxamide derivatives and incorporated long alkylamino groups, and found that the compounds containing longer alkylamino chains are more potent than the substituents with the shorter alkyl chains. Among the synthesized compounds, compounds 24a, 24b, and 24c showed the maximum potency with each MIC value of 25 µg/mL compared to the standard drug isoniazid with an MIC value of 0.1–0.39 µg/mL.[63] Semelkova*et al.* synthesized N-Benzyl pyrazine carboxamide derivative and screened the compounds for their anti-tubercular activity. Compound 25a, containing the electronegative group methyl group, and compound 25b, containing the electron-withdrawing group chlorogroup, showed anti-tubercular activity with the MIC value of each 12.5 µg/mL compared to the standard drug isoniazid with an MIC value of 0.2 µg/mL.[64,65] Jandourek *et al.* designed and synthesized a pyrazine carboxamide derivative incorporating various substituted benzyl amino derivatives at the 3-position. Compounds 26a and 26d contain the electron-withdrawing group trifluoromethyl, whereas 26b and 26c, containing the electron-releasing group methyl and the amino group, has shown better or equal potent activity with the MIC value of 12.5 µg/mL, 12.5µg/mL, 1.56µg/mL, and 6.25 µg/mL, respectively, compared to the standard drug PZA with an MIC value of 12.5 µg/mL.[66] Shaik *et al.* designed and synthesized chloropyrazine carboxamide derivatives incorporating different alkyl groups containing several electron-donating and electron-withdrawing groups, along with some derivatives containing heterocyclic rings. Compound 27a, containing electron-withdrawing chloro group at ortho and para position in the phenyl group, showed 16 times improved activity over the reference compound PZA (MIC value of 50.80 µg/mL) with an MIC value 25.51 µg/mL of whereas compound 27b containing electron releasing group 3,4,5-trimethoxyphenyl group showed 17 times higher potency with MIC value of 23.89 µg/mL.[67] Ramesh *et al.* designed and synthesized

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Table 2: MIC values of pyrazine-containing derivatives

Compound code	Structural features	MIC value	Reference
12	Carboxamide and pyrazine	6.25 and 3.13	Dolezal *et al*. (2008)
13	Carboxamide and pyrazine	<2 and 4	Dolezal *et al*. (2009)
14	Carboxamide and pyrazine	1.56 and 3.13	Zitko *et al*. (2013)
15	Benzylcarboxamide and pyrazine	6.25 and 3.13	Servusová *et al*. (2013)
16	Carboxamide and pyrazine	3.13	Zitko *et al*. (2015)
17	Benzylamine and pyrazine	6.25	Jandourek *et al*. (2014)
18	Long alkyl group and pyrazine	25	Semelkova *et al*. (2015)
19	Benzylamine and pyrazine	12.5	Semelkova *et al*. (2017)
20	Benzylamine and pyrazine	12.5, 1.56, 6.25, and 12.5	Jandourek *et al*. (2017)
21	Aromatic ketone and pyrazine	25.51 and 23.89	Shaik *et al*. (2020)
22	Pyridine carboxamide and pyrazine	1.59	Ramesh *et al*. (2024)

MIC: Minimum inhibitory concentration

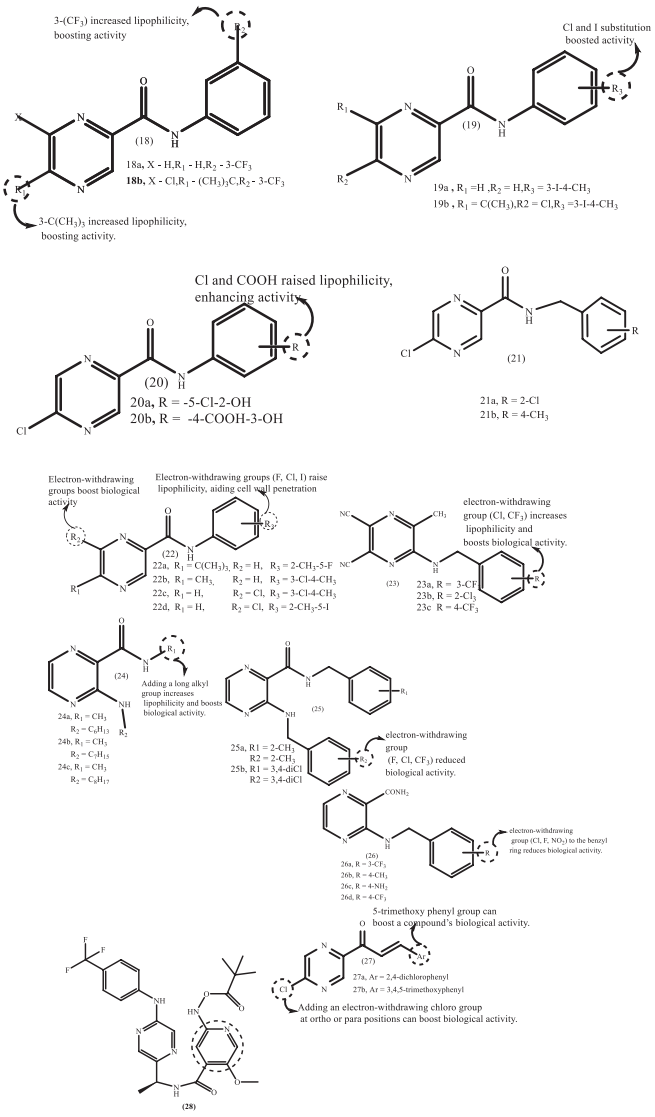


Figure 6: Compounds containing ortho-meta-substituted pyrazine

pyrazine derivatives for their anti-tubercular activity. Compound 28, containing the electron-withdrawing group

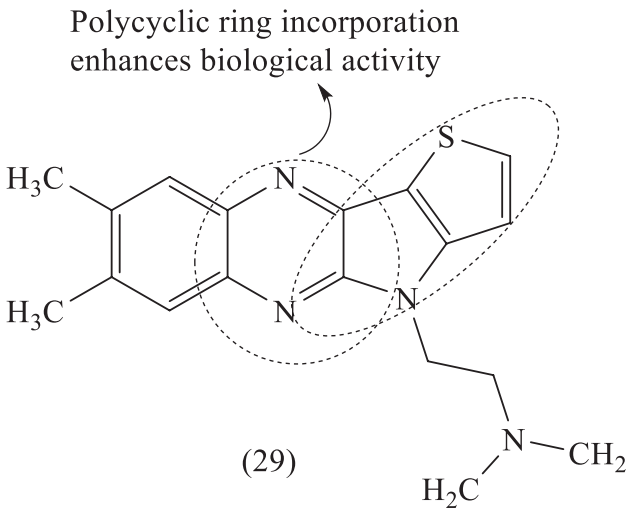
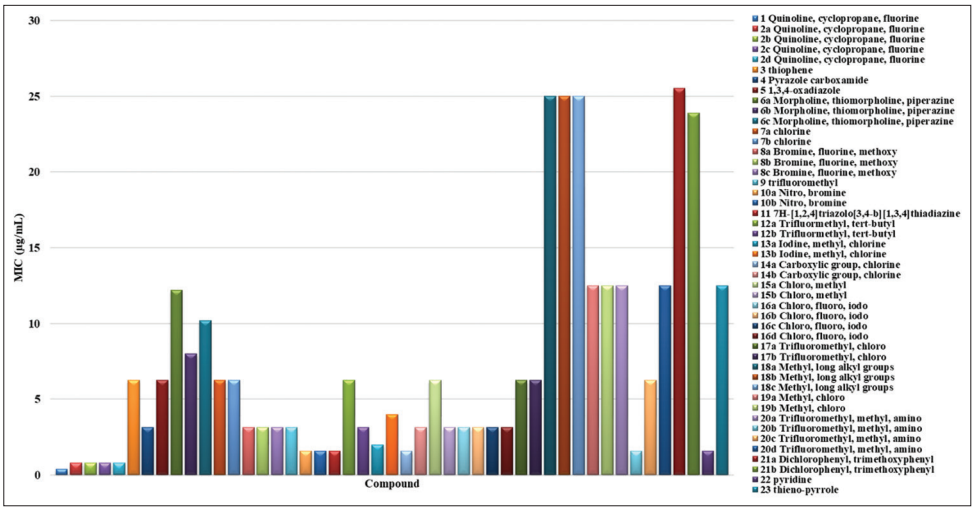


Figure 7: Compound containing a polycyclic ring fused to pyrazine

fluoro, showed the maximum potency with the MIC value of 1.59 µg/mL, whereas the standard drug used is isoniazid with the MIC value of 0.1 µg/mL.[68] The compounds' chemical structures are shown in Figure 6, and the MIC value with the structural feature is shown in Table 2.

Polycyclic ring fused pyrazine derivatives

Sadykhov *et al*. designed and synthesised a pyrrole and quinoxaline hybrid compound that contains thiophene and N, N-dimethyl ethyl substitution. They evaluated the compounds for their anti-tubercular activity. Compound 29 showed the highest potency amongst all the synthesised compounds with an MIC value of 12.5 µg/mL compared to the standard drug isoniazid with an MIC value of 0.06 µg/mL.[69,70] The compounds' chemical structures are shown in Figure 7, and the MIC value with the structural feature is shown in Table 3.



Graph 1: Minimum inhibitory concentration value of the novel compounds reported from the year 2010 to 2025 as an anti-tubercular agent

Table 3: MIC values of pyrazine-containing derivatives

Compound code	Structural features	MIC value	Reference
23	Polycyclic ring containing pyrazine	12.5	Sadykhov *et al.* (2024)

MIC: Minimum inhibitory concentration

CONCLUSION

The pyrazine nucleus has emerged as a highly promising class of compounds due to its structural simplicity, pharmacophoric versatility against *M. tuberculosis*, for the resistant strains as well. The SAR studies suggest that the substitutions of the pyrazine ring with electron-withdrawing groups or heterocyclic moieties significantly enhance the anti-tubercular potency, often surpassing the standard drugs like PZA in terms of their MIC values. The effect of the various substitutions, such as electron-withdrawing, electron-releasing groups, and the various heterocyclic rings, has been compiled in Graph 1 for thorough understanding. Advances in synthetic methodologies and computational modelling have further enabled rapid lead optimization and drug-likeness profiling. Taken together, the SAR evidence reviewed here indicates that the pyrazine nucleus is not merely a structural motif but a modifiable platform capable of addressing emerging resistance mechanisms in TB therapy.

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