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Review Article

Recent Progress on Pyrazole Scaffold-Based Antimycobacterial Agents

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New and reemerging infectious diseases will continue to pose serious global health threats well into the 21st century and according to the World Health Organization report, these are still the leading cause of death among humans worldwide. Among infectious diseases, tuberculosis claims approximately 2 million deaths per year worldwide. Also, agents that reduce the duration and complexity of the current therapy would have a major impact on the overall cure rate. Due to the development of resistance to conventional antibiotics there is a need for new therapeutic strategies to combat *Mycobacterium tuberculosis*. Subsequently, there is an urgent need for the development of new drug candidates with newer targets and alternative mechanism of action. In this perspective, pyrazole, one of the most important classes of heterocycles, has been the topic of research for thousands of researchers all over the world because of its wide spectrum of biological activities. To pave the way for future research, there is a need to collect the latest information in this promising area. In the present review, we have collated published reports on the pyrazole core to provide an insight so that its full therapeutic potential can be utilized for the treatment of tuberculosis. In this article, the possible structure–activity relationship of pyrazole analogs for designing better antituberculosis (anti-TB) agents has been discussed and is also helpful for new thoughts in the quest for rational designs of more active and less toxic pyrazole-based anti-TB drugs.

Keywords: Anti-tubercular / Medicinal chemistry / *Mycobacterium tuberculosis* / Pyrazole / Synthesis / Tubercle bacillus

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Introduction

Human tuberculosis (TB) is caused by infection with members of the *Mycobacterium tuberculosis* complex, which includes *M. tuberculosis* (*Mtb*) itself, *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium caprae*, *Mycobacterium microti*, *Mycobacterium pinnipedii*, and *Mycobacterium canettii* [1, 2]. *Mtb* also known as the white plague was identified by Robert Koch in 1882 [3]. Highly complex interactions of *Mtb* with the human host have been studied intensively ever since. Despite these efforts, many critical gaps in our knowledge remain, precluding successful control of the TB pandemic [4, 5]. TB

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Abbreviations: AIDS, acquired immunodeficiency syndrome; **DHP,** dihydropyridine; **DOS,** directly observed therapy short-course; **DOTS,** directly observed treatment short course; **EMB,** ethambuthol; **GI,** growth inhibition; ***h*CB,** human cannabinoid; **HIV,** human immunodeficiency virus; **INH,** isoniazid; **LJ medium,** Löwenstein–Jensen medium; **LRP,** luciferase reporter phage; **LTBI,** latent tuberculosis infection; **MABA,** microplate alamarBlue assay; ***M. africanum*,** *Mycobacterium africanum*; ***M. smegmatis* (MS),** *Mycobacterium smegmatis*; ***M. bovis* (MB),** *Mycobacterium bovis*; ***M. caprae*,** *Mycobacterium caprae*; ***M. fortuitum* (MF),** *Mycobacterium fortuitum*; **MDR,** multidrug-resistant; **MDR-TB,** multi-drug resistant tuberculosis; **MIC,** minimal inhibitory concentration; ***Mtb*,** *Mycobacterium tuberculosis (M. tuberculosis)*; **NSAID,** non-steroidal anti-inflammatory drug; **NTM,** non-tubercular mycobacterial; ***NR-MTB*,** non-replicating *Mycobacterium tuberculosis*; **PZA,** pyrazinamide; **RIF,** rifampicin; ***R-Mtb*,** replicating *Mycobacterium tuberculosis*; **RTK,** receptor tyrosine kinase; **SAR,** structure–activity relationship; **SI,** selectivity index; **SLD,** second-line drugs; **STM,** streptomycin; **TB,** tubercle bacillus; **WHO,** World Health Organization; **XDR-TB,** extensively drug-resistant tuberculosis.

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usually attacks the lungs but can also spread through air. Many people become symptom-free carriers of the TB bacteria. Although common and deadly in the third world, TB was almost non-existent in the developed world, but has been making a recent resurgence. Certain drug-resistant strains are emerging and people with immune suppression such as acquired immunodeficiency syndrome (AIDS). Most infections in humans result in an asymptomatic, latent infection and about 1 in 10 latent infections eventually progresses to active disease. New infections caused by *Mtb* are registered to occur at a rate of about one per second [6].

TB has been one of the deadliest diseases over the past few decades affecting nearly one-third of the world's population [7] with new infection occurring at 1% of population each year [8]. According to World Health Organization (WHO), in 2011 there were 8.7 million new cases of TB (13% co-infected with human immunodeficiency virus [HIV]) and 1.4 million people died from TB including 1 million HIV negative people [9]. It is estimated that 1.7 million people died in 2009 due to TB only [9], while new 9.4 million cases of TB were identified in the same year, of which the majority were in Asia and Africa. It is thought that the rates of new TB infections and deaths *per capita* have probably been falling globally for several years now. However, the total number of new TB cases is still slowly rising due to population growth and poor health. Between 1990 and 2005, TB incidence in Africa accounted for an estimated 78% of TB cases among HIV-positive people worldwide. The largest number of TB cases occurs in Asia, which in 2009 accounted for an estimated 56% of the global total. However the estimated incidence *per capita* in sub-Saharan Africa is around twice that of South-East Asia. The countries of Eastern Europe are also facing a serious epidemic; there were over 150000 new cases in Russia alone in 2009. TB is not only a problem in low- and middle-income countries. For example, there were 11545 new cases reported in the USA in 2009. In the UK, TB has been dubbed the disease that has never gone away, with 9040 new cases of TB reported in the UK in 2009 [10]. Although the UK's national rate is very low in comparison with most of the world, London has become one of the world's TB hotspots. In parts of London, TB rates are ten times higher than national rate and some countries of the former Soviet Union. About 10% of people with TB in London are likely to be co-infected with HIV [11].

Of the people who died of TB worldwide in 2008, it is estimated that 400000 were infected with HIV. TB is the leading cause of death among HIV-infected people in Africa. When someone is infected with TB, the likelihood of them becoming sick with the disease is increased many times if they are also HIV positive. The different cultures of the TB and HIV communities raise many challenges in achieving an effective and productive partnership. TB services are geared toward chronic-care services with simple and standardized technical procedures, while HIV/AIDS services are clinically oriented and tend to be more individual-patient-oriented [12]. Therefore, people with latent TB are increasingly becoming infected with HIV, and many more are developing active TB because HIV is weakening their immune system. People who are co-infected with both HIV and latent TB have an up to 800 times greater risk of developing active TB disease and becoming infectious compared to people not infected with HIV [12].

It has been established that nitrogen-containing heterocyclic compounds play an important role in designing new class of structural entities for medicinal applications. Among nitrogen heterocyclic compounds, pyrazole (**1**) and its derivatives are significant because of their wide spectrum of biological activities and their presence in naturally occurring compounds. They are widely used as core motifs for a large number of compounds for various applications such as catalysis, agro-chemicals, building blocks of other compounds, and in medicine. Pyrazoles which belong to the family of azoles were first described by Knorr in 1833 [13]. They may be considered to have been derived from pyrrole by substitution of its 2-carbon by nitrogen atom, i.e., they are aromatic dinitrogen heterocyclic compounds. In pyrazoles, with one basic nitrogen and neutral nitrogen, the aromatic nature arises from the four π electrons and the unshared pair of electrons on the –NH nitrogen. Care should be taken in specifying which nitrogen is basic, since in unsymmetrical derivatives resonance prevents one from isolating a specific compound (**1**). 1*H*-Pyrazole (**1**) and its derivatives normally do not occur in nature probably due to difficulty in a constructing a N–N bond by living organisms. The dihydro pyrazoles are called pyrazoline and three of them are possible depending on the position of the double bond. These are 4,5-dihydro-3*H*-pyrazole (**2**), 4,5-dihydro-1*H*-pyrazole (**3**), and 2,3-dihydro-1*H*-pyrazole (**4**) [14] (Fig. 1).

Pyrazoles and their derivatives have attracted much attention due to their diverse application in agrochemical, pharmaceutical, and chemical industries [15]. They possess a wide range of bioactivities [15, 16], including antiviral [17], anti-inflammatory [18], anticonvulsant [19], anticancer [20], insecticidal [21], antibacterial [22], antifungal [23, 24], antispasmodic [25], analgesic [26], antihyperglycemic [27], hypoglycemic [28], antineoplastic [29], antidepressive [30], antipyretic [31] activities, CNS regulants [32], and selective enzyme inhibitory activities [33]. It has been found that these compounds have hypoglycemic activity, and are also known as inhibitors and deactivators of liver alcohol dehydrogenase and oxido-reductases [34]. It has been shown *in vivo* that some of the pyrazole derivatives have appreciable antihypertensive activity [35]. These compounds also exhibit properties such as

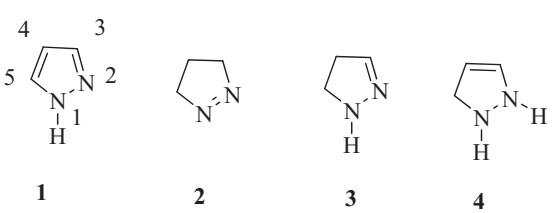


Figure 1. Structures of pyrazole and pyrazolines.

cannabinoid human cannabinoid (*h*CB1) and *h*CB2 receptor, inhibitors of p38 kinase, CB1 receptor antagonists [36, 37]. It has also been found that, various substituted pyrazoles are used as chelating and extracting reagents for many metal ions [38]. Moreover, these derivatives are used as starting material for the construction of condensed heterocyclic systems and represent an interesting template for combinatorial chemistry [38, 39].

To list a few pyrazole derivatives that are already booming in the market, some of them are as follows: celecoxib (**5**, COX-2 selective non-steroidal anti-inflammatory drug [NSAID]), antipyrine/phenazone (**6**, analgesic, NSAID and an antipyretic drug), analgin/metamizole (**7**, analgesic, antispasmodic (spasm reliever) and antipyretic), allopurinol/zyloprim (**8**, hyperuricemia and xanthine oxidase inhibitor), phenylbutazone (**9**, analgesic, NSAID and an antipyretic drug), oxyphenbutazone (**10**, NSAID) [1], pirazole (**11**, NSAID) [40], pyrazofurin (**12**, adenosine kinase) [41], ramifenazone (**13**, analgesic, antipyretic and anti-inflammatory agent) [42], indisteron (**14**) [43], apixaban (**15**, anticoagulant), fipronil (**16**, insecticide), rimonabant (**17**, anorectic antiobesity drug) [44] and many more are already in market (Fig. 2).

Therefore, the synthesis of pyrazole and its derivatives has received an increasing attention to synthetic organic chemists and biologists. Some reviews on involvement of pyrazole nucleus as different biological agents are available in the literature [45], but no review was published on pyrazole nucleus as anti-tubercular agents. A large amount of effort has been invested in the past decade to develop pyrazole-based compounds as modulator of antituberculosis (anti-TB), which is active on different clinically approved therapeutic targets showing excellent therapeutic potency. It is still a challenge for the pharmaceutical chemist to develop more effective and less toxic agents to treat signs and symptoms of TB disorders. By looking into the importance of this therapeutic area we decided to collect the published anti-TB data on pyrazole (from 2000 to date), the indispensable anchor in medicinal chemistry. In this review, we have attempted to shed light and compile published reports on pyrazole derivatives along with structure–activity relationship (SAR) and some opinion on different approaches to help the medicinal chemists in designing future generation potent yet safer anti-TB agents.

Symptoms and complications of tuberculosis

There are no symptoms associated with inactive TB. This means that someone may have acquired the TB bacteria and yet show no signs or symptoms of infection. Symptoms only appear when the TB infection becomes active. Symptoms develop gradually, and it may take many weeks before you notice that something's wrong and see your doctor. Although the TB bacteria can infect any organ (e.g., kidney, lymph nodes, bones, and joints) in the body, the disease commonly occurs in the lungs [46, 47].

Common symptoms include:

- coughing that lasts longer than 2 weeks with green, yellow, or bloody sputum,
- weight loss,
- fatigue,
- fever,
- night sweats,
- chills,
- chest pain,
- shortness of breath,
- loss of appetite.

The occurrence of additional symptoms depends on where the disease has spread beyond the chest and lungs. For example, if TB spreads to the lymph nodes, it can cause swollen glands at the sides of the neck or under the arms. When TB spreads to the bones and joints, it can cause pain and swelling of the knee or hip. Genitourinary TB can cause pain in the flank with frequent urination, pain or discomfort during urination, and blood in the urine [48].

Diagnosing tuberculosis

TB can be diagnosed by three major methods:

- skin test,
- chest X-ray,
- sputum culture test.

A tuberculin skin test allows a doctor to check your immune response to the TB bacteria. It is a test that is used for detecting infection with the TB bacteria. It is given to people who have been exposed to patients with active, contagious TB or to those in whom reactivation of TB is suspected. Skin testing involves an injection in the forearm 2 or 3 days later, a physician will "read" the test. If it is positive, indicated by a hard and swollen region at the site of injection, this means that your body is been infected by the TB bacteria. It does not necessarily mean that you have active TB – the TB may be inactive. Chest X-rays can also be performed, and sputum samples can be analyzed in the lab. In these cases, the results are used to rule out or confirm active TB. Your doctor may also suggest other tests to confirm a diagnosis or to check for TB in other parts of your body. Sputum is a thick fluid produced in the lungs and in the airways leading to the lungs. For the sputum culture test, sputum of the person is taken and cultured in a suitable medium. If there is no bacterial growth, the culture is negative. After 2–3 weeks, a colony of bacteria can be seen if the person is infected with *M. tuberculosis* [49].

Main tuberculosis drugs in clinical use

Anti-TB drugs are classified into five groups based on evidence of efficacy, potency, drug class, and experience of use [50]. All first-line anti-TB drug names have a standard three-letter

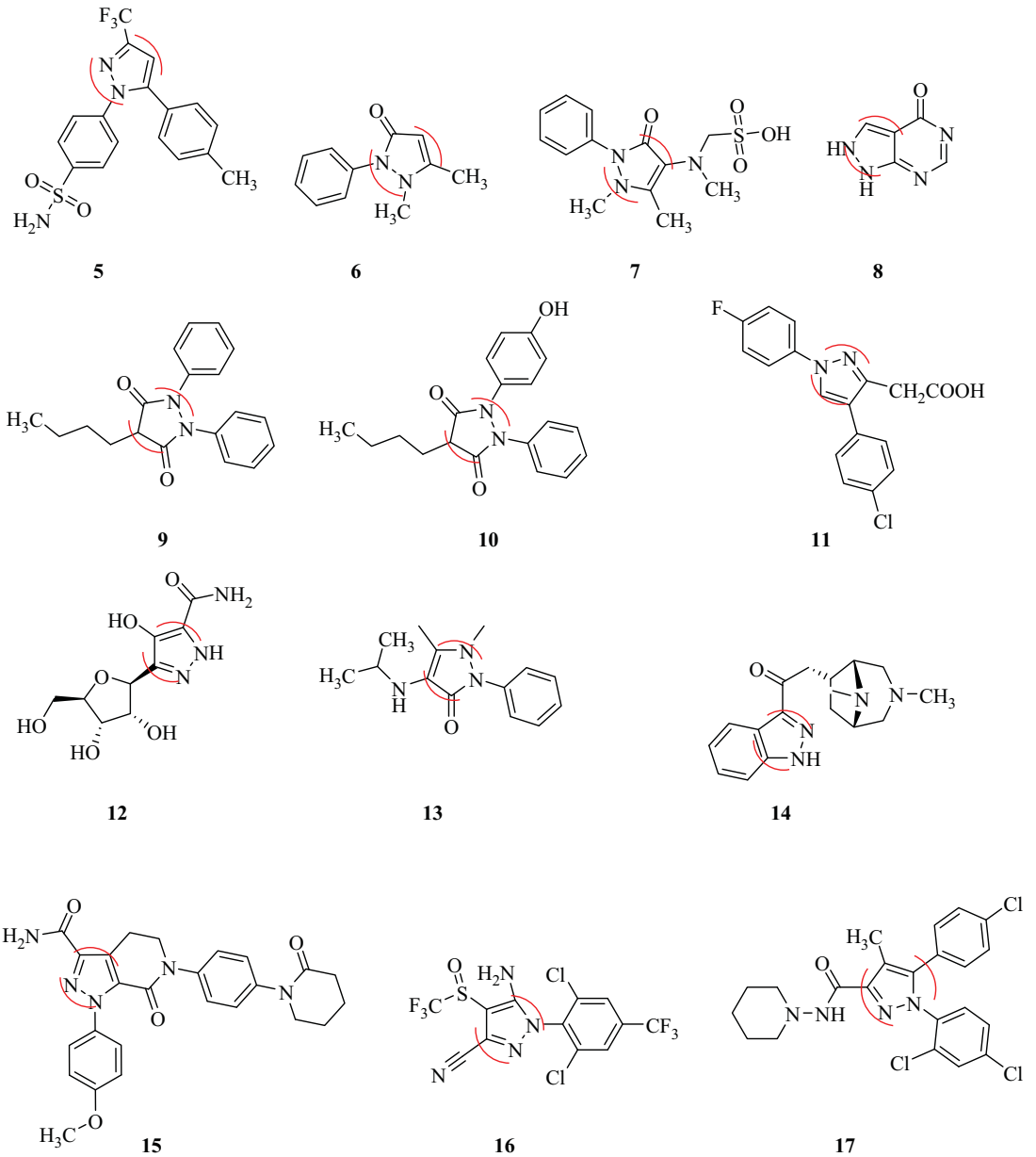
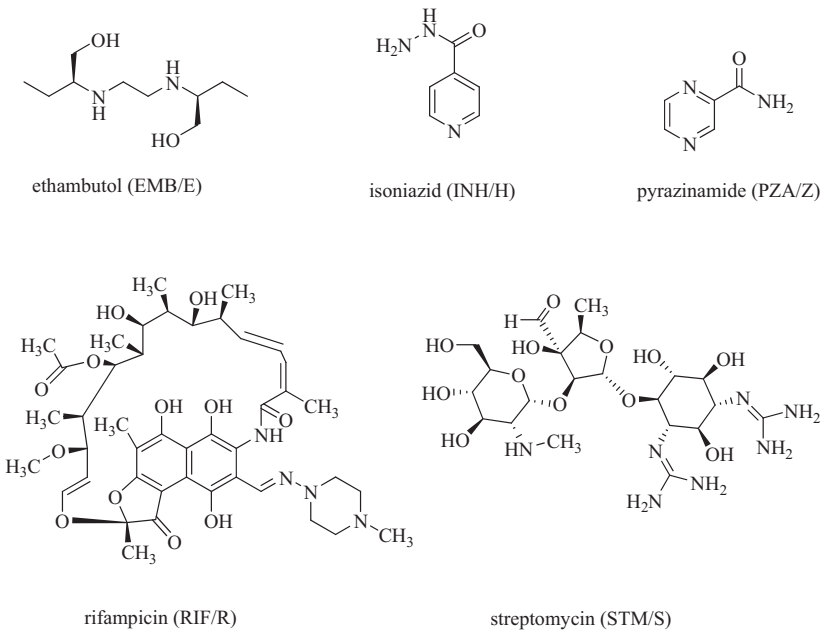


Figure 2. Pyrazole, a multifunctional nucleus.

and/or a single-letter abbreviation. The first-line anti-TB drugs are ethambutol (EMB/E) [51, 52], isoniazid (INH/H) [53, 54] pyrazinamide (PZA/Z) [55–58], rifampicin (RIF/R) [59–61], and streptomycin (STM/S) [62, 63] given for 6 months (Fig. 3).

Second-line drugs (SLDs) are those that are less effective than the first-line or have some side effects. The unavailability of a drug in many developing countries also makes it SLD. If the treatment fails as a result of bacterial drug resistance, or intolerance to one or more drugs, SLDs are used. The SLDs are only used to treat disease that is resistant to first-line therapy (i.e., for extensively drug-resistant tuberculosis (XDR-TB) or multidrug-resistant tuberculosis (MDR-TB)) [64]. Further, the SLDs are divided into six classes; these are (i) aminoglycosides (amikacin/AMK, kanamycin/KM), (ii) polypeptides (capreomycin, viomycin, enviomycin), (iii) fluoroquinolones (ciprofloxacin/CIP, moxifloxacin/MXF, levofloxacin), (iv) thioamides (prothionamide, ethionamide), (v) cycloserine, and (vi) terizidone and *p*-aminosalicylic acid/PAS/P (Fig. 4).

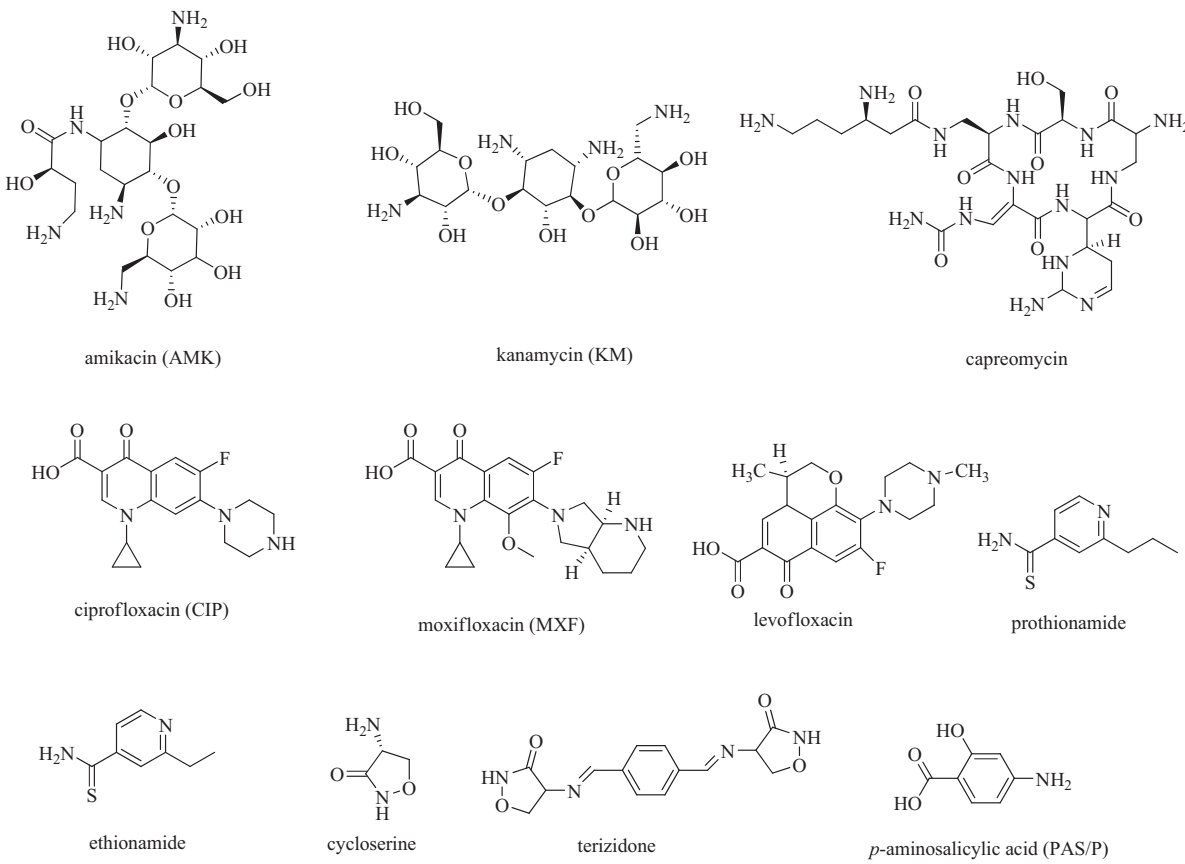
The third-line drugs are either not very efficient or their effectiveness is not yet established. This includes rifabutin,



ethambutol (EMB/E) isoniazid (INH/H) pyrazinamide (PZA/Z)

rifampicin (RIF/R) streptomycin (STM/S)

Figure 3. First-line anti-TB drugs.



amikacin (AMK) kanamycin (KM) capreomycin

ciprofloxacin (CIP) moxifloxacin (MXF) levofloxacin prothionamide

ethionamide cycloserine terizidone *p*-aminosalicylic acid (PAS/P)

Figure 4. Second-line anti-TB drugs.

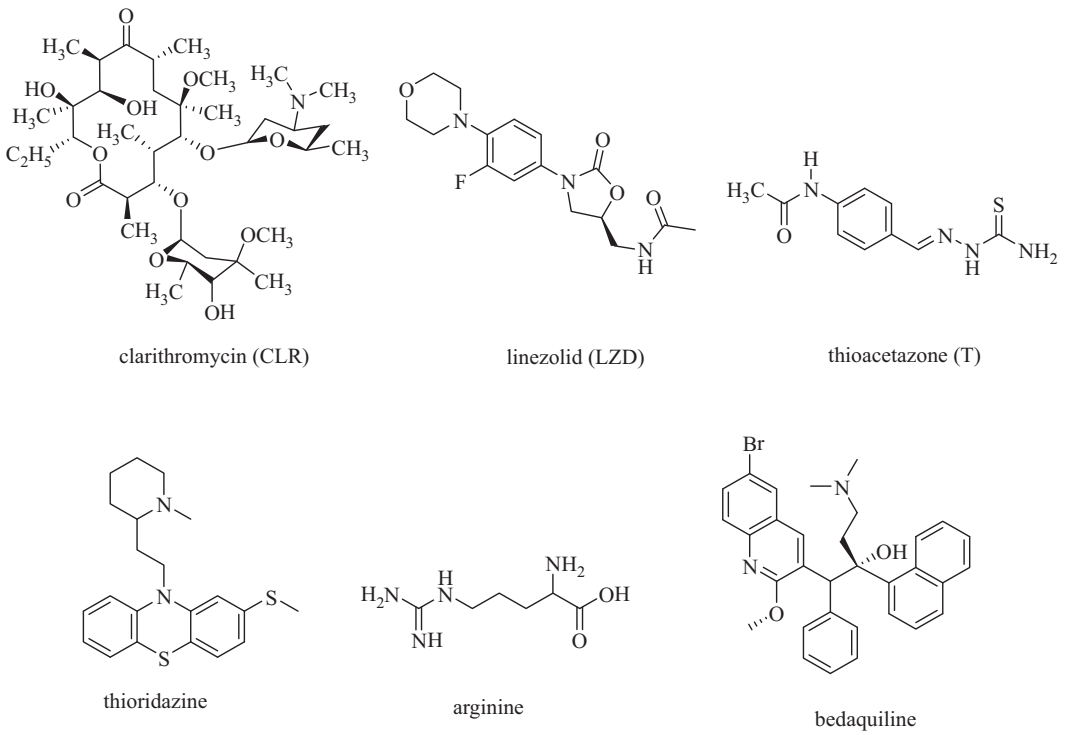


Figure 5. Third-line anti-TB drugs.

macrolides: e.g., clarithromycin (CLR); linezolid (LZD); thioacetazone (T); thioridazine; arginine; vitamin D; bedaquiline (Fig. 5). Rifabutin is effective, but is not included on the WHO list because for most developing countries, it is impractically expensive [65].

WHO-recommended directly observed treatment short course (DOTS) anti-TB therapy involves the administration of four drugs: INH, RIF, PZA, and EMB or SM. Treatment with these so called first-line drugs is carried out initially over 2 months, leading to the destruction of bacteria in all growth stages, after which treatment continues with RIF and INH alone for 4 months, where any residual dormant bacilli are eliminated by RIF and any remaining RtF-resistant mutants are killed by INH [66, 67].

Pyrazole and its derivatives: Structural requirements for anti-TB activity

From collected published data, it is found that pyrazole nucleus substituted at all position with varied substituents has produced potent anti-TB activity. The 1st position of pyrazole may be unsubstituted or substituents may vary from aryl groups. Among them, pyrazole with 2-Cl, 3-Cl, 4-Cl, 4-CH_3, and 2-Cl-5-SO_2 at phenyl substituents showed excellent anti-TB activity. Similarly, 2nd position may be substituted with phenyl substituents with fluorine, trifluoro, and nitro, showed good activity. The 3 or 4-position of the nucleus may be substituents are more because of the conjugation and substituents may range. At the 3rd position of pyrazole with functional groups like halogen, methyl, methoxy, and nitro substituents in phenyl ring enhances the anti-TB activity. Also at the 4th position presence of 1-(*p*-methoxyphenyl)-2-propenone, 4-hydrophenyl-2-aminopyrimidine, *p*-chlorobenzoxyl, piperidine, or methyl piperidine showed excellent activity. Finally, 5th position with –CF_3, napthyl ring, phenyl ring with –Cl, –CH_3 of pyrazole shows good anti-TB activity (Fig. 6).

Pyrazole derivatives for treatment of tuberculosis

Yadlapalli and co-workers reported synthesis of diarylpyrazole ligated dihydropyrimidines (DHPs) derivatives by utilizing Biginelli reaction and evaluated for their *in vitro* anti-TB activity against *Mtb* $H_{37}Rv$ using microplate alamarBlue assay (MABA). Compounds **18a**,**b**, with a minimal inhibitory concentration (MIC) value of 0.125 and 0.25 μg/mL respectively, were showed most potent in the series. SAR studies revealed, compounds with chlorine (–Cl) were more potent **18a**,**b**, when compared to compounds with other substituents like H [68]. Fluoro substituted pyrazolylpyrazoline derivatives

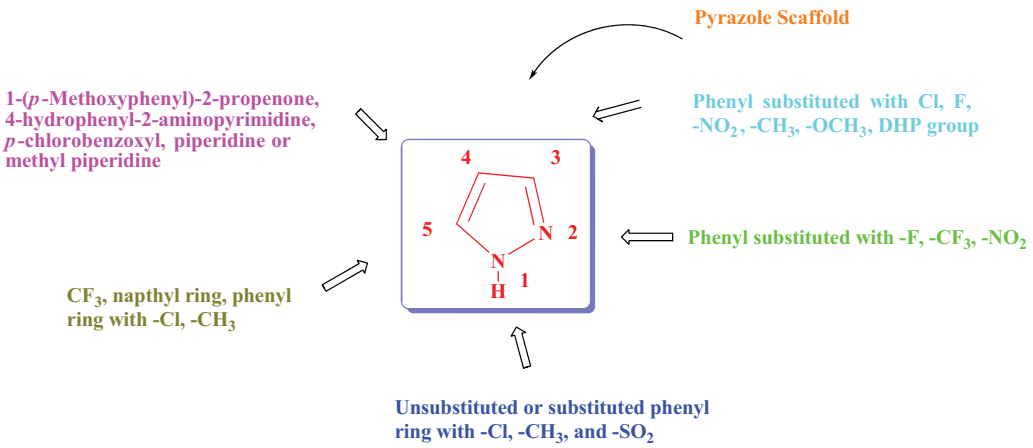


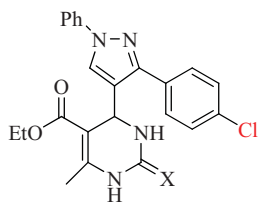
Figure 6. Structural requirements around the pyrazole nucleus for anti-TB activity.

were synthesized under microwave irradiation and anti-TB activity was screened at 250 μg/mL against *Mtb H37Rv* stain by using Löwenstein–Jensen (L–J) medium. Compounds **19a–d** possess excellent activity (i.e., 90, 91, 96, and 94% at 250 μg/mL) against *Mtb H37Rv*. SAR study reveals, the presence of electron withdrawing group (4-F, 4-Br) at R_1 position and $-CF_3$ group at *meta* position of aryloxy ring illustrated anti-TB activity. The presence of bromo group at R_1 position and fluoro group at *ortho*, *meta*, and *para* positions of aryloxy ring showed enhanced anti-TB activity [69] (Fig. 7).

Benzoxazole-based pyrazoline derivatives were synthesized by Rana et al., and evaluated for *in vitro* anti-TB studies against *Mtb H37Rv* and *MDR-TB* strains. Some of the compounds displayed potent activity (MIC = 0.625–25 μg/mL) and few compounds were found better than standard INH against MDR-TB (MIC = 3.25 μg/mL). Compounds **20b** and **21** were found potent as good as INH against *Mtb H37Rv*, while **20a** was found more potent than INH against *MDR-TB* strain [70].

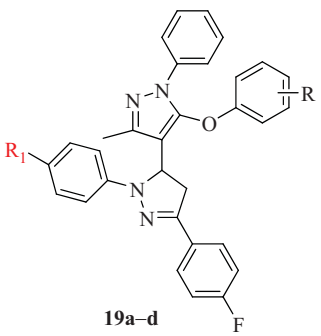
Horrocks and co-workers described the synthesis of 3-(4-chlorophenyl)-4-substituted pyrazole derivatives and tested for anti-TB activity against *Mtb H37Rv* strain. The introduction of electron-withdrawing groups like pyridine (**22a**), nitro (**22b**), or chloro (**22c**) group in the phenyl ring resulted in very good antimycobacterial activity, while the electron-releasing groups like hydroxyl, methoxy, or amino showed poor activity. SAR study reveals, the electron-withdrawing groups in the phenyl ring are important for antimycobacterial activity [71] (Fig. 8).

Pathak and co-workers investigated pyrazole bearing 2-azetidinones and 4-thiazolidinones derivatives as anti-TB agents. Best activity against *Mtb H37Rv* was observed for the 2-OH substituted azetidinone derivative (**23**), which is slightly less potent than the standard drug RIF, followed by the 3,4-$(OCH_3)_2$ substituted hydrazone derivative (**24**); the 2- and 4-OH substituted thiazolidinone derivatives **25a,b** showed better anti-TB activity. SAR study reveals, the substituents like



18a,b

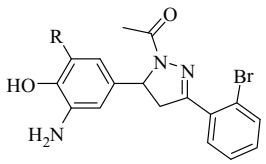
a) X = S; MIC(*Mtb*) = 0.125 μg/mL
b) X = O; MIC(*Mtb*) = 0.25 μg/mL



19a–d

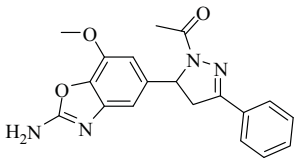
a) R = 3-CF_3, R_1 = 4-F; % inhibition (*Mtb*) = 90%
b) R = 3-CF_3, R_1 = 4-Br; % inhibition (*Mtb*) = 91%
c) R = 4-F, R_1 = 4-Br; % inhibition (*Mtb*) = 96%
d) R = 2-F, R_1 = 4-Br; % inhibition (*Mtb*) = 94%

Figure 7. Structures of the compounds **18** and **19**.



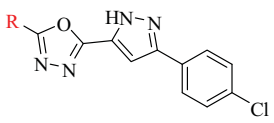
20a,b

a) R = OCH_3, MIC(*Mtb*) =12.5 μg/mL
MIC(*MDR-TB*) = 3.25 μg/mL
b) R = H, MIC(*Mtb*) = 0.625 μg/mL
MIC(*MDR-TB*) = 12.5 μg/mL



21

MIC(*Mtb*) = 0.625 μg/mL
MIC(*MDR-TB*) = 6.25 μg/mL



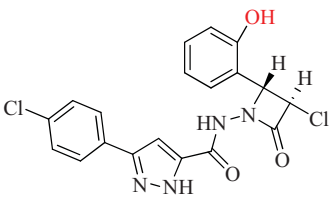
22a-c

a) R = pyridine; MIC(*Mtb*) = 0.42 μg/mL
b) R = 4-$NO_2C_6H_4$; MIC(*Mtb*) = 0.5 μg/mL
c) R = 2-ClC_6H_4; MIC(*Mtb*) = 0.6 μg/mL

Figure 8. Structures of the compounds **20–22**.

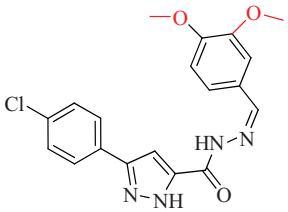
–OH, –Cl, $–OCH_3$ enhance the anti-TB activity [72]. A series of 4-substituted pyrazoles have performed and tested for *in vitro* antimycobacterial activity against *Mtb H37Rv*. Substitution of 1-(*p*-methoxyphenyl)-2-propen-1-one at the 4th position of the pyrazole ring produced the most active molecule (**26**) with a IC_{50} of 0.47 μM. Of compound **27** with a 4-hydroxyphenyl-2-aminopyrimidine at 4th position of the pyrazole ring, concentrations of 0.88 and 0.47 μM are required for 90 and 50% inhibition, respectively [73] (Fig. 9).

Manikannan and co-workers performed synthesis of isomeric pyrazoles derivatives and antimycobacterial activity has been screened for their *in vitro* activity against *Mtb H37Rv*. Compounds with a *p*-nitrophenylthio ring (**28a** and **29**) in the pyrazole ring showed excellent anti-TB activity and also all the aryl ring carrying *p*-chloro group (**28b**) or when the substituents are *p*-chlorophenylthio and cyclohexylthio groups (**28c**). SAR study reveals, *p*-chlorobenzoyl moiety at the C_4 of the pyrazole ring has found remarkable antimycobacterial activity in some of the pyrazole compounds [74]. Recently, Monga et al., have investigated pyrazoline inhibit the strong anti-TB activity against *Mtb H37Rv*. Pyrazoline derivative substituted with electron-donating methoxy groups at the



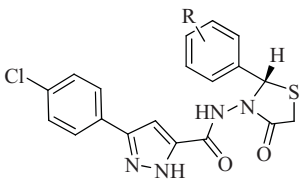
23

MIC(*Mtb*) = 0.35 μg/mL



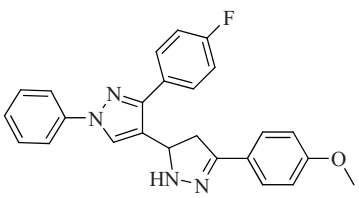
24

MIC(*Mtb*) = 0.37 μg/mL



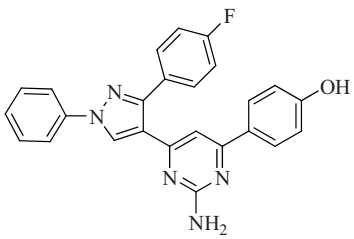
25a,b

a) R = 2-OH; MIC(*Mtb*) = 0.5 μg/mL
b) R = 4-OH; MIC(*Mtb*) = 0.39 μg/mL



26

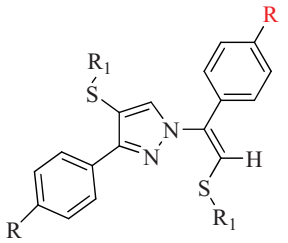
IC_{50} (*Mtb*) = 0.47 μM



27

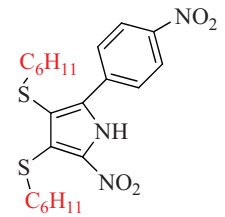
IC_{90} (*Mtb*) = 0.88 μM
IC_{50} (*Mtb*) = 0.47 μM

Figure 9. Structures of the compounds **23–27**.



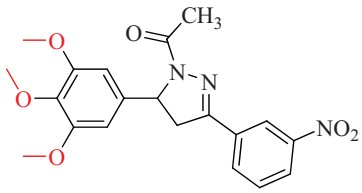
28a,b

a) R = NO_2, R_1 = C_6H_4; MIC(*Mtb*) = 0.78 μg/mL
b) R = Cl, R_1= C_6H_4; MIC(*Mtb*) = 1.56 μg/mL
c) R = *p*-C_6H_4, R_1 = *p*-C_6H_4; MIC(*Mtb*) = 1.56 μg/mL



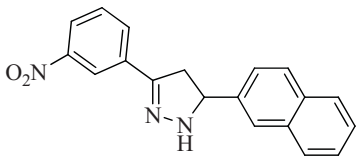
29

MIC(*Mtb*) = 1.56 μg/mL



30

MIC(*Mtb*) = 12.5 μg/mL
% Inhibition = 99%



31

MIC(*Mtb*) = 62.5 μg/mL
% Inhibition = 99%

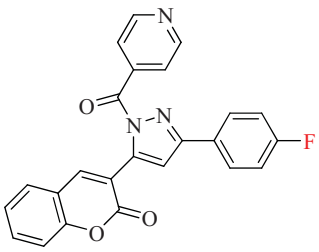
Figure 10. Structures of the compounds **28–31**.

meta/*para* position of the phenyl rings (**30**) displayed highest activity against *Mtb* with MIC value of 12.5 μg/mL. Compound possessing naphthyl ring attached to C-5 of the pyrazoline ring (**31**) showed better anti-TB activity, having MIC value of 62.5 μg/mL (Fig. 10) [75].

Aragade and co-workers studied a series of pyrazole derivatives containing INH and coumarin moieties, evaluated anti-TB activity against *Mtb* $H_{37}Rv$ using Resazurin MIC assay. Compound **32** containing a 4-fluoro group at C-3 phenyl ring of pyrazole nucleus has showed promising antimycobacterial agent. From SAR, the presence of electron withdrawing groups such as –F, –Cl, and –NO_2 on the phenyl ring of pyrazole moiety at C-3 position may be attributed for enhanced antimycobacterial activity. The presence of 4-fluoro group compound displayed relatively higher inhibitory activity [76]. Chovatia and co-workers performed the synthesis of diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives and screened for activity against *Mtb* $H_{37}Rv$ at a concentration of 6.25 μg/mL in BACTEC 12B medium using the ALAMAR radiometric and results were compared with the standard drug RIF. Compound with methoxy (**33**) and methyl (**34**) group in the phenyl ring attached to pyrazole, showed 86% inhibition at 6.25 μg/mL (Fig. 11) [77].

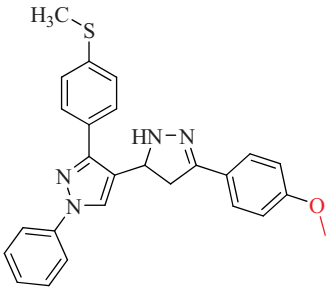
Amino-4,5-dihydro-1*H*-pyrazolylmethanethione derivatives were also reported as anti-TB agents by Ali et al. Compounds were tested for their antimycobacterial activity *in vitro* against *Mtb* $H_{37}Rv$ and INH resistant *Mtb (INHR-Mtb)* using BACTEC method and results were compared with INH. Among the synthesized compounds, **35** was found most active agent against *Mtb* $H_{37}Rv$ and *INHR-Mtb* with MIC of 0.43 μM. When compared to INH, compound **35** was found 1.62- and 26.41-fold more active against *Mtb* and *INHR-Mtb*, respectively. Compounds with chloro substituted phenyl group showed higher anti-TB activity [78]. Namrata and co-workers identified benzyloxy phenyl butenyl azoles as anti-TB agents. The compounds possess potent anti-TB activities with MIC comparable to the standard drugs. Especially, compound **36** exhibited significant anti-TB activity with MIC value as low as 0.61 μg/mL, comparable to many standard drugs. SAR study reveals, substituent on the 4-benzyloxy ring did not display any significant change on the anti-TB activity. Compounds with imidazole and benzimidazole moieties showed promising anti-TB activity with MIC values from 1.56 to 12.5 μg/mL (Fig. 12) [79].

Trivedi and co-workers carried out synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives and assessed antimycobacterial activities against *Mtb* using MABA. Compounds **37a–d** exhibited best results (1.2 μg/mL) when compared with first-line drugs such as INH and RIP. SAR study reveals, the presence of 2-



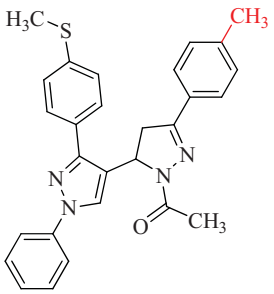
32

MIC(*Mtb*) = 0.625 μg/mL
% Inhibition = 80.09%



33

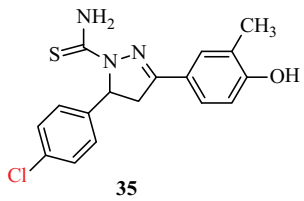
% Inhibition = 86%



34

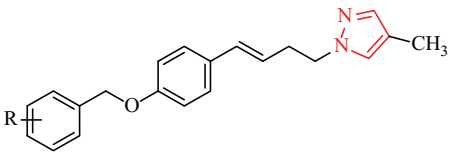
% Inhibition = 86%

Figure 11. Structures of the compounds **32–34**.



35

MIC(*Mtb*) = 0.43 μg/mL
MIC(*INHR-Mtb*) = 0.43 μg/mL



36

MIC(*Mtb*) = 0.61 μg/mL

Figure 12. Structures of the compounds **35** and **36**.

chloro-, 3-chloro-, 4-methyl-, and 2-chloro-5-sulfonyl substituents markedly enhances anti-TB activity [80]. In continuation of their work, author synthesized DHP bearing 1*H*-pyrazole ring substituted at C-4 position as anti-TB agents. Compound **38** was found most active compound *in vitro* with MIC of 0.02 μg/mL against *Mtb* and was more potent than INH. SAR study reveals, compounds with carbethoxy substituents at C-3 and C-5 position of DHP ring exhibited higher antimycobacterial activity than the compounds with acetyl substituents at C-3 and C-5 position [81]. Same author reported, pyrazole ring bearing 4-DHP derivatives were prepared and screened for their *in vitro* antimycobacterial activity at 6.25 μg/mL against *Mtb* $H_{37}Rv$ strain. Compound **39** with 4-nitro group at the 3-aryl substituent on pyrazole nucleus along with carbomethoxy group at C-3 and C-5 position of DHP ring was the most potent, while compounds **40a,b** with 4-fluoro and 4-bromo groups, respectively, at the 3-aryl substituent on pyrazole nucleus along with carbethoxy group at C-3 and C-5 position of DHP ring were the most potent. SAR reveals, DHP derivatives bearing alkyl ester group at C-3 and C-5 position along with electron-withdrawing groups at the 4th position of 3-aryl substituents on pyrazole nucleus have exhibited comparatively higher antimycobacterial activity probably due to their higher lipophilicity (Fig. 13) [82].

A series of 3*H*-indeno[1,2-*c*]pyrazole-2-carboxamide analogues were reported and evaluated for *in vitro* antimycobacterial activity against *Mtb* and *INHR-Mtb*. The compounds **41a,b** were found active against *Mtb* at a MIC of 0.78 μM comparable to that of INH. The compounds **41a,b** were found to be active against *INHR-Mtb* at a MIC of 0.78 and 3.12 μM, respectively. When compared with INH, the compound **41a** was 16-fold more active while compound **41b** was 4-fold more active against *INH-Mtb*. SAR study reveals, 3-substituted compounds with electron withdrawing groups such as 4-fluorophenyl, 4-pyridyl produced more inhibitory and 2-chlorophenyl produced moderate inhibitory activity while the electron releasing groups such as 4-methoxyphenyl, 3,4-dimethoxyphenyl showed less inhibitory activity. The 4-fluoro substitution on *N*-aryl group showed maximum inhibitory activity as compared to 4-bromo and 3-chloro-4-fluoro substitution [83]. In continuation of their work on anti-TB drugs, authors reported 3*H*-indeno[1,2-*c*]pyrazole-2-carbothioamide analogues inhibit activity against *Mtb* and *INH-Mtb*. Compound **42** was found to be active against *Mtb* and

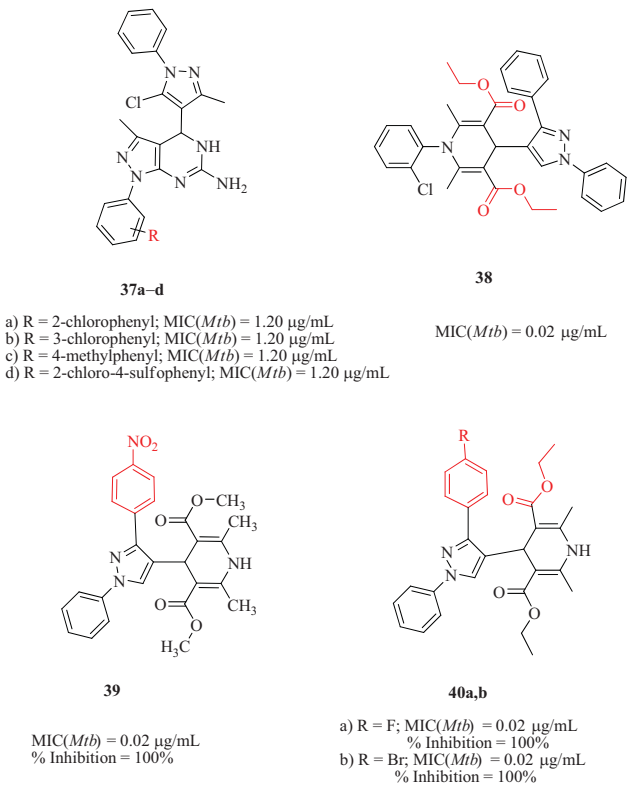


Figure 13. Structures of the compounds **37–40**.

INH-Mtb at a MIC of 3.12 and 6.25 μM, respectively. When compared with INH the compound **42** was fourfold less active than INH against *Mtb* and twofold more active than INH against *INHR-Mtb*. From SAR concluded same as above, 3-substituted compounds with electron withdrawing groups such as 4-fluorophenyl produced more inhibitory activity than 2-chlorophenyl and 2-pyridyl substitution [84]. In another article, same author reported synthesis pyrazole-2-carboxamide analogues as anti-TB agents. Compound **43** was active against *Mtb* and *MDR-TB* at a MIC of 0.83 and 3.32 μM, respectively. When compared with INH, the compound **43** was less potent against *Mtb*, while 13.6-fold more active against *MDR-TB*. SAR study reveals, 4-chlorophenyl substitution on *N*-aryl group showed maximum inhibitory activity than 4-nitrophenyl substitution or when there was no substitution (Fig. 14) [85].

Castagnolo and co-workers developed novel rigid pyrazolone derivatives and evaluated as inhibitors of *Mtb*. The presence of a chlorine atom on the N_1-phenyl ring caused an improvement in the activity with respect to non-halogenated and compound bearing *N*-Me-piperazine (**44a**) and morpholine moieties (**44b**) proved to be very active with MIC 4 μg/mL. SAR study reveals, the presence of piperidine or Me-piperidine moieties showed detrimental for the activity [86]. Pyrazoline derivatized carbazole derivatives were reported as anti-TB agents by Taj et al. Compounds with electron donating groups **45a–c** have exhibited excellent inhibition with MIC less than 5 μg/mL. The anti-TB activity is attributed to the presence of electron donating atoms/groups viz., –Cl, –OH, and $–OCH_3$ through mesomeric effect appended to the pyrazoline moiety (Fig. 15) [87].

Gunasekaran and co-workers performed the synthesis of 2-aryl-1*H*-3-pyrazolone derivatives by one-pot, four-component sequential reactions using *p*-toluenesulfonic acid in water. The synthesized compounds were tested for *in vitro* antimycobacterial activity against *Mtb*. The compound **46** displayed the maximum potency with a MIC of 1.6 μM against *Mtb*, being 2.94 and 4.75 times more active than CIP and EMB, respectively. SAR study reveals, aryl ring at C-11 bearing electron withdrawing groups such as halogens and nitro show greater activity than that with electron-releasing groups, viz.

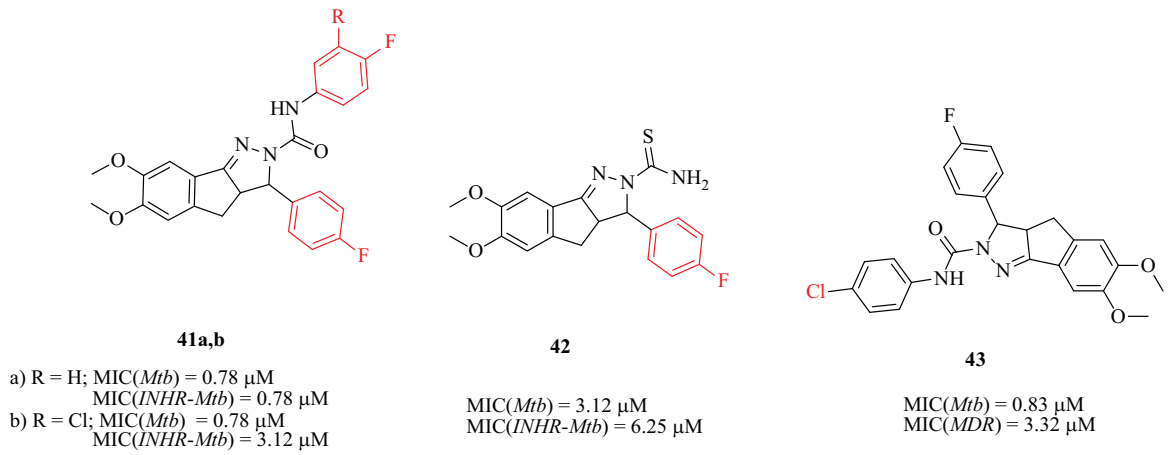
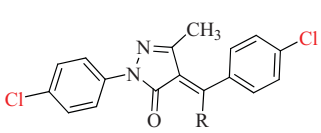
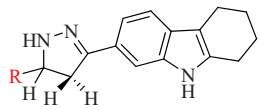


Figure 14. Structures of the compounds **41–43**.



44a,b

a) R = *N*-Me-piperazine; MIC(*Mtb*) = 4 μg/mL
b) R = morpholine; MIC(*Mtb*) = 4 μg/mL



45a–c

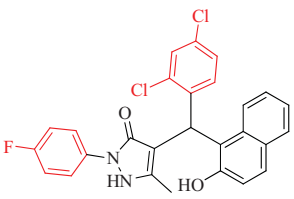
a) R = *p*-chlorophenyl; MIC(*Mtb*) = 3.5 μg/mL
b) R = *p*-hydroxyphenyl; MIC(*Mtb*) = 3.5 μg/mL
c) R = methyl; MIC(*Mtb*) = 3.5 μg/mL

Figure 15. Structures of the compounds **44** and **45**.

methoxy, methyl, and isopropyl [88]. A series of isonicotinoyl-3-(pyridin-2-yl)-pyrazole derivatives reported for evaluating their antimycobacterial activity toward a strain of *Mtb H$_{37}$Rv* and a strain of *Mtb* H$_4$. Compounds **47a–e** showed good anti-TB activity at 8 μg/mL against *Mtb H$_{37}$Rv* and a strain of *Mtb* H$_4$ strains. The inhibition of INH release through a possible stabilizing effect of a hydrogen bond between the hydroxyl group in the *ortho* position on the 3-aryl residue and the nitrogen atom at the 2-position in the pyrazoline cycle of these compounds (Fig. 16) [89].

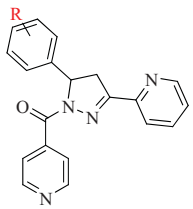
Silva and co-workers performed the synthesis of isonicotinoyl-4,5-dihydropyrazole derivatives and tested *in vitro* anti-TB activity against INH-susceptible *Mtb H$_{37}$Rv*, *INHR-Mtb* isolates and non-tuberculous mycobacteria. Among the synthesized compounds **48a,b** exhibited antimycobacterial activity in the same range as INH for the susceptible H$_{37}$Rv strain and higher activity than INH against resistant strains such as RGH101, RGH102, and RGH103. Compound **48c** is not the most active against *Mtb H$_{37}$Rv*, it is the only compound having activity against all the tested *INH-R Mtb*. SAR study reveals, compounds with trifluoromethyl-substituted pyrazolines (**48a** and **48c**) were more active than their analogs trichloromethyl-substituted pyrazolines [90]. Chobe et al., disclosed the synthesis of pyrano-[2,3-*c*]-pyrazole derivatives and compounds were investigated against *Mycobacterium smegmatis*, *Mycobacterium pheli*, and *Mtb* species. The activity may centered and enhanced on the presence of naphthaleno moiety containing dihalo group, i.e., chloro and fluoro as well as the methoxy group in these compounds. The strain *Mtb* was observed to be resistant toward compound **49** (MIC, 100 μg/mL), was found to be effective growth inhibitors of this strain. The presence of hydroxyl, dihalo, and methoxy group shows moderate activity and these compounds might have relevance in imparting the growth inhibition (GI) activity against *Mtb* (Fig. 17) [91].

A series of 3-amino- pyrazole derivatives were synthesized and screened for *Mtb H$_{37}$Rv* inhibition assay. Compound **50** shows (MIC < 1 μg/mL, IC$_{50}$ = 0.004 mg/mL), **51** (MIC = 0. 4 μg/mL), **52** (MIC < 1μg/mL), and **53** (MIC = 0. 4μg/mL) excellent anti-TB activity [92–96]. Pandit and Dodiya reported pyrazole-quinazolinone hybrid analogs as anti-TB agents. Compounds containing *ortho* directing substitutions of chloro, methyl, and methoxy in basic skeleton led to increase in anti-TB activity. Compounds **54a–e** exhibited excellent anti-TB activity with percentage inhibition of 96, 90, 94, 93, and 92, respectively at a MIC value of 6.25 μg/mL. SAR study reveals, compounds containing electron-releasing groups, such as –CH$_3$ and –OCH$_3$ groups at *ortho* position and electro negative group –Cl at *ortho* and *para* positions led to increase in activity, while electro negative group at *meta* position led to increased activity owing to bulky group, ring strain increased and made compound less stable and active [97]. Khanage and co-workers described the synthesis of pyrazole



46

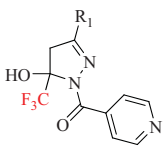
MIC(*Mtb*) = 1.6 μM



47a–e

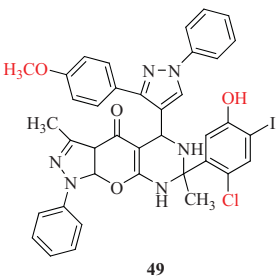
a) R = H; MIC(*Mtb, Mtb H$_4$*) = 8 μg/mL
b) R = 2-Cl; MIC(*Mtb, Mtb H$_4$*) = 8 μg/mL
c) R = 3-Cl; MIC(*Mtb, Mtb H$_4$*) = 8 μg/mL
d) R = 4-Cl; MIC(*Mtb, Mtb H$_4$*) = 8 μg/mL
e) R = 2-CH$_3$; MIC(*Mtb, Mtb H$_4$*) = 8 μg/mL

Figure 16. Structures of the compounds **46** and **47**.



48a–c

a) R = H; MIC(*Mtb*) = 0.77 μM
MIC(RGH101) = 12 μM
b) R = 4-MePh; MIC(*Mtb*) = 2.23 μM
MIC(RGH101) = 8.94 μM
c) R = 2-furyl; MIC(RGH101) = 38.46 μM
MIC(RGH102) = 38.46 μM
MIC(RGH103) = 19.23 μM



49

MIC(*Mtb*) = 100 μg/mL

Figure 17. Structures of the compounds **48** and **49**

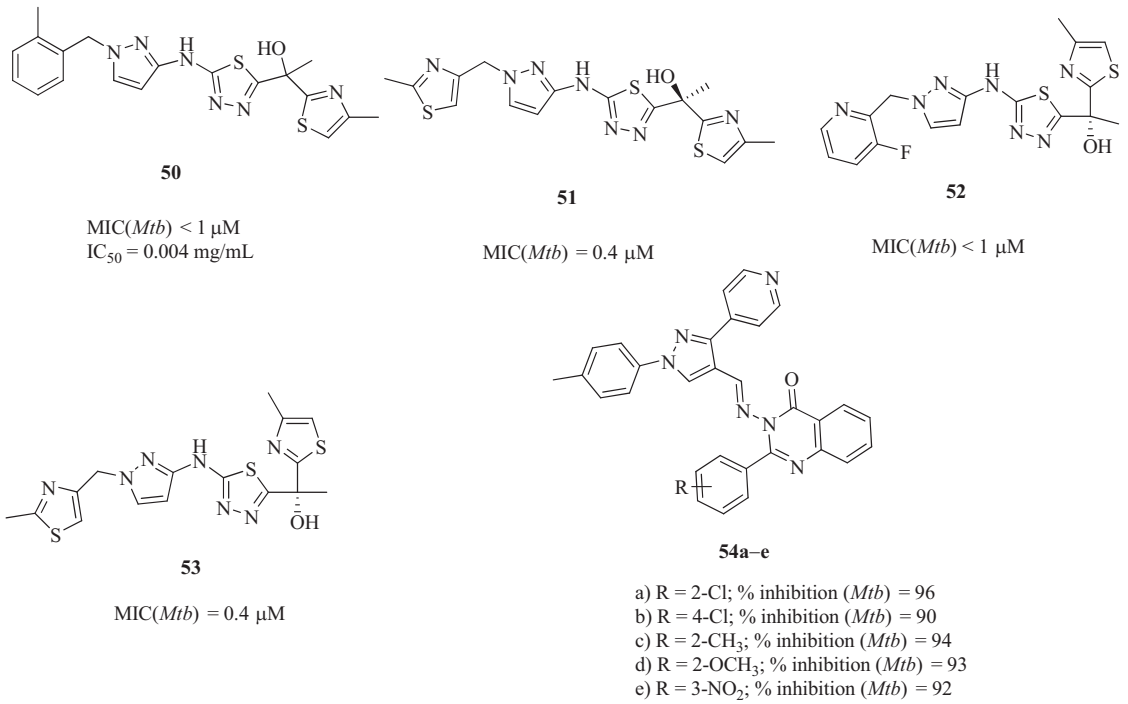


Figure 18. Structures of the compounds **50–54**.

derivatives containing 1,2,4-triazole derivatives evaluated for *in vitro* anti-TB and *Mtb* $H_{37}Rv$ (ATCC27294) in BACTEC 12B medium using a broth micro-dilution assay. 4-Chloro, 3-nitro, 4-methoxy, and 2-chloro substituted compounds were found to be anti-mycobacterial agents at MIC value of 6.25 μg/mL (Fig. 18) [98].

Conclusion and future aspects

Due to the unusual structure and chemical composition of the mycobacterial cell wall, effective TB treatment is difficult, which makes many antibiotics ineffective and hinders the entry of drugs. With approximately 33% of infection, tuberculosis is still the second most imperative infectious disease worldwide. Although pyrazoles are rarely found in natural products, numerous pyrazole derivatives display a broad spectrum of pharmaceutical and agrochemical activities, and they have also been successfully applied in other fields. This has given rise to search for molecules acting on a novel target or a multi-targeted combination therapy. With the increase in the number of new compounds screened against mycobacteria, the opportunity exists to develop a novel drug for the cure and complete eradication of TB. This review is an attempt to address the vistas of anti-TB potential of strategically placed pyrazole scaffold in medicinal chemistry and drug development. Information provided in this manuscript can be found useful for further investigations on this scaffold in order to harness its optimum anti-TB potential. To further optimize the full potential of pyrazole compounds, the SAR-based study will likely continue to play an important role. It is highly likely that optimized pyrazole compounds with excellent potency and little side effects will continue to be created. Some of these pyrazole compounds will undoubtedly be used as first and second-line anti-TB therapeutic agents in the near future. Further investment in developing fundamental genetic systems and more accurate models of human disease would significantly facilitate TB drug discovery efforts in the long term, in particular enabling robust validation of novel targets.

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The authors have declared no conflict of interest.

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