

Review

Antitubercular drug discovery: the molecular modification as promise tool

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Tuberculosis is a chronic infectious disease caused by etiologic agent *Mycobacterium tuberculosis*. “It has been reported that rates of incidence is increasing and each year around 8.9-9.9 million new cases are detected”. The current TB therapy presents several problems such as long-term therapy, drug resistance, no-compliance to therapy and few therapeutic sources to treat the disease. The discovery of new drugs safer and more efficient is urgent. The molecular modification is an important tool that allows discovering new compounds that can be win these challenges. In this work, we summarize the main strategies useful to obtain a new anti-tubercular drug.

Keywords: Tuberculosis; antitubercular drugs; new drugs; molecular modification.

INTRODUCTION

Tuberculosis (TB) represents the chronic infectious disease that more kills adults worldwide (Trabulsi and Alterthum, 2005). Between century XVII and XVIII, TB was known as “great white plague” or “tífise. TB was responsible for more than one billion deaths between 1700 and 1900 overcoming any other disease (Daniel et al., 1994).

One of the first studies about TB was performed by Robert Koch (1843-1810) who isolates and cultivates the etiologic agent *Mycobacterium tuberculosis* (MTB). These results revolutionized the story of Bacteriology and Medicine and this mycobacteria came to be known as Koch's bacillus. Structurally, MTB presents a complex cell wall composed of long chain fatty acids (with 60-90 carbon atoms known as mycolic acids), glycolipids, peptidoglycan and proteins (Trabulsi and Alterthum, 2005).

TB is transmitted by coughing of a sick individual. The droplets containing expectorated MTB are inhaled and penetrates host lung beginning the infection process (WHO, 2011; Trabulsi and Alterthum, 2005). After MTB infect human lung, an inflammatory process with macrophages activation initiates leading to pro-

inflammatory cytokines production such as interleukin IL-6, IL-12, IL-1B, INF- γ (Flynn et al., 2011). The alveolar macrophages infected for MTB produce the Mcl-1 antiapoptotic protein, responsible to interrupt apoptosis of infected macrophages by regulation of mitochondrial membrane, preventing the release of cytochrome c and enzymes responsible for DNA degradation (Sly et al., 2003).

The Immune response acts against MTB allowing other cells to be recruited for infected side to interrupt bacterium growth leading to granuloma formation. The granuloma is an organized structure by immune cells, formed through antigenic response, which can be observed in latent and active tuberculosis. The granuloma is niche for the bacillus. In this state there is reduction of metabolism regarding immune response activity leading to a quiescence state. This MTB dormant is known as latent TB that unlike of active TB is not characterized as an infectious disease (Russell, 2011; Flynn, 2011).

Immunosuppressive conditions compromise the efficiency of the immune system allowing the reactivation of previously dormant bacilli, leading individual to develop active TB, usually decades after initial infectious. It has been reported that the risk to develop active TB in co-infected individual with HIV is 100 times when compared to only TB infected

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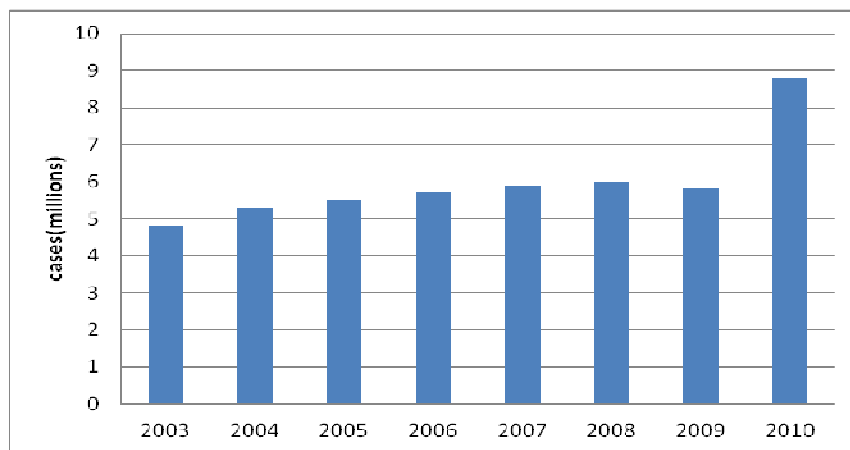


Figure 1. Number of new tuberculosis cases by WHO 2010 and 2011.

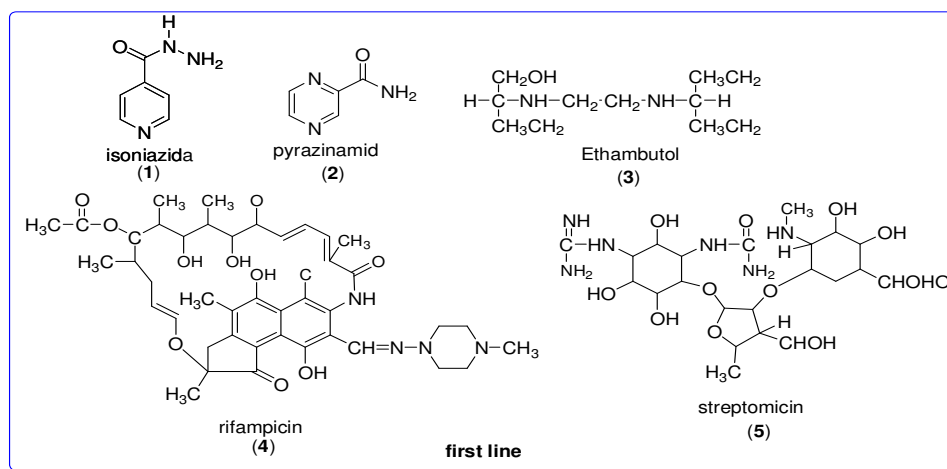


Figure 2. Chemical structures of first and second line antitubercular drugs.

individuals (Havir and Barnes, 1999; Pitchenik et al., 1988). Patients with AIDS are more susceptible to develop opportunistic infections by atypical mycobacteria as *Mycobacterium avium*, *M. kansasii*, *M. fortuitum* e *M. chelonae*. Furthermore, TB induces AIDS development in HIV-positive patients by stimulating cytokines productions and reduction of CD4 + T cells (Trabulsi and Alterthum, 2005).

Nowadays TB represents the second cause of death worldwide. The World Health Organization (WHO) estimates that almost one third of the population is infected with MTB. Furthermore, the number of new TB cases is increasing according WHO reports (Figure 1). Current drug therapy presents several problems such as side effects of drugs, resistance of MTB and comorbidity. Access to adequate health care in developing countries is another problem for the tuberculosis therapy (Cegielski and McMurray, 2004). The BCG vaccine (*Bacilo Calmette-Guérin*), an attenuated *Mycobacterium Bovis*, is used by more than a century but its efficiency is questionable (WHO, 2010; WHO,

2011; Haydel, 2010; Smith et al., 2009).

All these problems associated with multidrug-resistance justify the research of new safer and more efficient drugs with reduce adverse side effects allowing adequate compliance by patients.

Multidrug-resistant tuberculosis (MDR-TB)

MTB drug resistance occurs through two different ways: (1) Multidrug-resistant tuberculosis (MDR-TB)-resistance at least two first-line antibiotics, isoniazid and rifampicin; (2) Extensively drug-resistant TB (XDR-TB), is defined as resistant to first and second line drugs (Figure 2) (Raviglione and Smith, 2007).

The resistance mechanism is still a mystery to science, but several biochemical events are required for MTB resistance such as: a) decrease in the intracellular concentration of antibiotics by altering the permeability of outer and inner membranes; b) mutation or modification of the cellular target; c) action of the drug

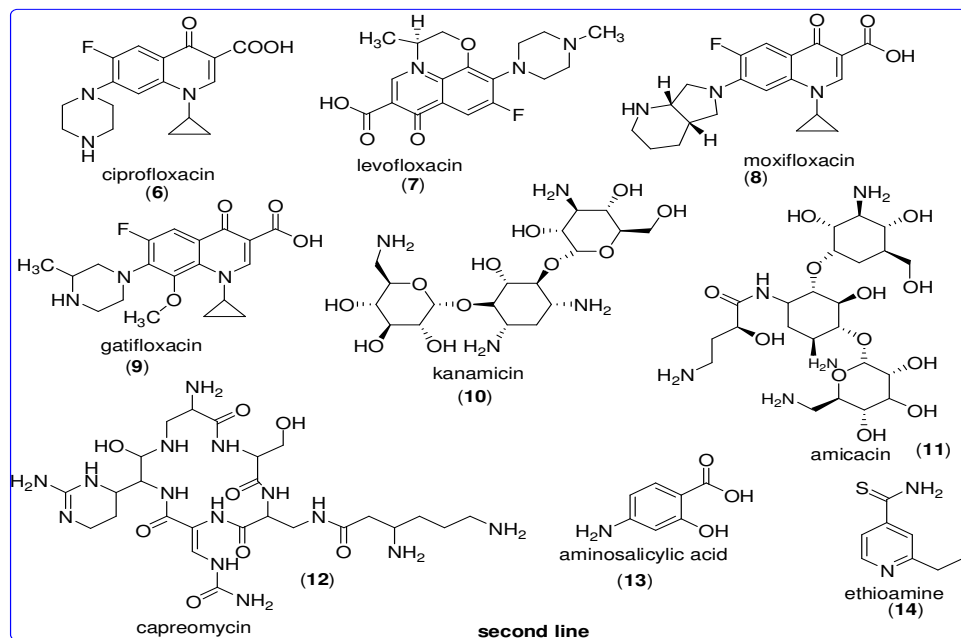


Figure 2b.

in another target. All these mechanisms performed by bacillus can lead to MDR-TB and XDR-TB state (Piddock et al., 2006; De Rossi et al., 2006).

Mutations in genes responsible for encoding enzymes recognized as targets, is the main factor to generate resistance to anti-TB drugs of first and second line. The specific interaction of rifampicin with β subunit of RNA polymerase enzyme inhibit transcription caused cell death. Mutations in the *rpoB* gene, which encodes the chain β enzyme, produce drug resistance by decreasing the interaction of rifampicin with the polymerase (Williams et al., 1998). Isoniazid is a prodrug that requires the *katG* gene product for its activation; this drug is active after metabolism of MTB catalase-peroxidase inhibiting the enoyl-ACP reductase enzyme, since it encoded by *inhA* gene; its resistance involves four genes: a) *katG*, which encodes the catalase-prexidase enzyme; b) *inhA* involved in mycolic acid biosynthesis; c) *ahpC*, that encodes alkyl hydroperoxide reductase C; and d) *oxyR*, regulator of oxidative stress (Zhang et al., 1992; Banerjee et al., 1994; Trabuasi and Alterthum, 2005). Ethambutol resistance is conferred by mutations in *embA*, *embB* e *embC* genes that encode enzymes involved in arabinano synthesis (Telenti et al., 1997). Pyrazinamide is a prodrug which is effective only against MTB inhibiting fatty acid synthesis. Its resistance occurs by mutations in *pncA* gene, encoding pirazianamidazes, enzyme that hydrolyzes the drug to make it active (Scorpio et al., 1997). The fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin and gatifloxacin) act on the enzymes responsible for the DNA topological conformation. Its resistance is conferred by mutations in the *gyrA*, *gyrB* genes, that encode respectively A and B subunits of

gyrase DNA (Takiff et al., 1994). Streptomycin acts in the inhibition of protein synthesis, and its resistance is involved with *rrs* gene, which encodes S12 rRNA, and *rpsL*, which encodes S12 ribosomal protein. The *rpl* also produces resistance to kanamycin and amikacin (Finken et al., 1993; Suzuki et al., 1998). Capreomycin resistance is caused by mutation in the *tlyA* gene (Maus et al., 2005). The ethioamine is an inhibitor of mycolic acid, responsible for cell wall synthesis and its resistance is due mutations in genes *inhA* (involved in mycolic acid biosynthesis) and *etha*, encoding monooxygenase flavin, which its responsible for activating etioamida (Banerjee et al., 1994). Aminosalicic acid resistance is due mutations in *thyA* genes, which encodes the synthase trimidalato enzyme, since its regulating intracellular concentrations of folate (Rengarajan et al., 2004).

MOLECULAR MODIFICATION

Molecular modification can enhance pharmacokinetics or pharmacodynamics aspects of the drugs. There are many strategy used by Medicinal Chemistry to design drugs using these strategies such as molecular hybridization, prodrug and bioisosterism. In this review, we suggest the application of this strategy to discivery new antitubercular drugs.

Molecular hybridization

The molecular hybridization is a rational approach to design new prototypes after fusion of pharmacophoric

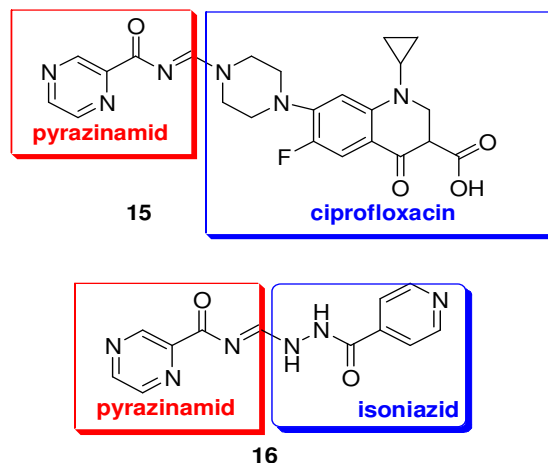


Figure 3. Molecular hybridization through pharmacophoric subunits fusion of antitubercular drugs.

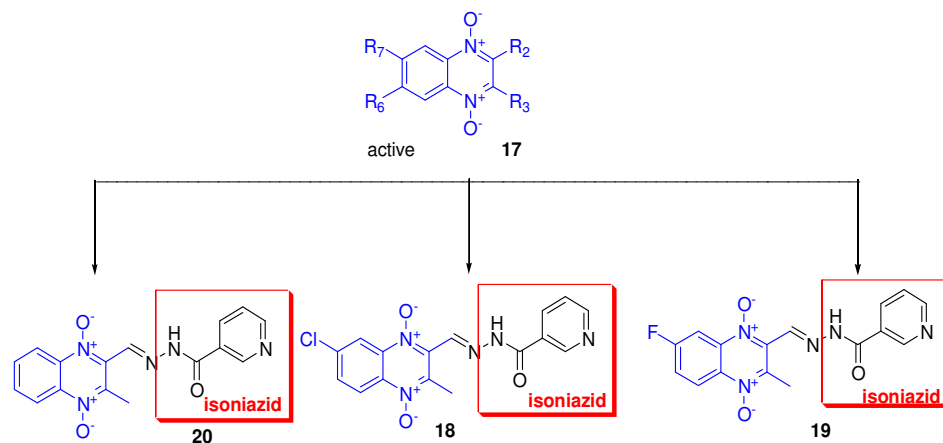


Figure 4. Quinoxaline-1,4-di-*N*-oxide derivatives as antitubercular drugs.

subunits that can be recognized by two or more biologic receptors (Veigas-Junior et al., 2007). This strategy has been used in TB drug discovery to increase the efficacy and reduce drug resistance. Imrarovský and co-workers combined through molecular hybridization the scaffold of three antitubercular drugs: isoniazid (first line), pyrazinamide (first line) and ciprofloxacin (second line). The compounds had great activity against MTB. The compounds **15** and **16** demonstrated with MIC = 0.78 $\mu\text{g/mL}$ and 0.1 $\mu\text{g/mL}$ respectively (Figure 3) (Imrarovský et al., 2007).

Quinoxaline-1,4-di-*N*-oxide has been used as scaffold for new hybrids compounds actives against MTB, (Ancizu et al., 2010). Recently, Torres and co-workers (2011) reported the antitubercular activity from the new quinoxaline-1,4-di-*N*-oxide derivatives (**17**) obtained by molecular hybridization with the first line drug, isoniazid. The authors demonstrated that halogens in position 7 increase the antitubercular activity. The compounds with chlorine (**18**) and fluorine (**19**) demonstrated IC_{50}

respectively 1.04 $\mu\text{g/mL}$ and 0.58 $\mu\text{g/mL}$, while the hydrogen substitution (**20**) demonstrated IC_{50} of 1.17 $\mu\text{g/mL}$ (Figure 4) (Torres et al., 2011).

Other interesting active prototype obtained by molecular hybridization was developed by Santos and co-workers (2009) between phthalimide subunit in the thalidomide and a dapsone. This scaffold was designed to be active against *M. leprae*, but it presented interesting activity against MTB with MIC of 3,9 $\mu\text{g/mL}$ (Santos et al., 2009).

Prodrug approach

In 1958, Albert called prodrug as “any compound that undergoes biotransformation prior to exhibit its pharmacological effects” (Albert, 1958). Lately, Haper (1959) try to improve this definition proposing the term latentation, which was defined as “the chemical modification of biologically active compound to form a new

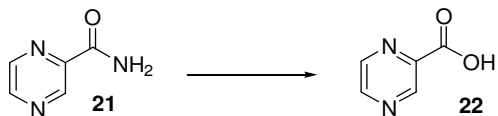


Figure 5. *In vivo* pyrazinamide conversion to pyrazinoic acid.



Figure 6. Esters and amides synthesized by Simões and co-workers (2009).

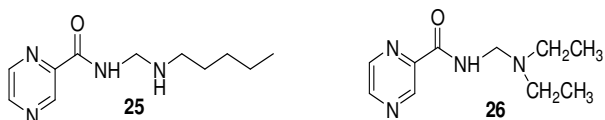


Figure 7. News compounds synthesized by Chung and co-workers (2009).

compound that, upon *in vivo* enzymatic attack, will liberate the parent compound" (Haper, 1959). Prodrug is classified into two main classes: bioprecursors and carrier prodrugs.

Bioprecursors is a molecular modification that generates new active substances that after metabolic reaction become active. The carrier prodrug after chemical or biological biotransformation releases the parental drug responsible for the biological activity and the carrier, generally without pharmacological effect (Silva et al., 2005).

Pyrazinamide (**21**) is a prodrug bioconverted by pyrazinamidases to active form pyrazinoic acid (**22**). This last one, decreases MTB growth (Figure 5) (Zhang, 2005).

Simões and co-workers (2009) studied the influence of carriers on pyrazinamide (**23**) and pyrazinoic acid (**24**), and they synthesized esters and amides compounds with higher lipophilicity than pyrazinamide. Posteriorly, the biological results have revealed that esters derivatives presented better activity against MTB than amides derivatives (Figure 6) (Simões et al., 2009).

An interesting study performed by Chung and co-workers (2008) about pyrazinamide carriers, led to a series of amino methylene analogs of pyrazinamide (**25** and **26**). They evaluated the influence of the size carbon chain size in these carriers and related these to biological activity. The authors discovered two new prototypes (**25** and **26**) as antitubercular drugs (Figure 7) (Chung et al., 2008).

Using the molecular hybridization, Mao and co-workers

(2007) synthesized the prodrug 5-(2,8-di(trifluoromethyl)quinolin-4-yloxymethyl)isoxazole-3-carboxylic acid ethyl ester (**29**). This compound was obtained through combination between mefloquine fragment (**27**) and isoxazolecarboxamide (**28**) (Mao et al., 2007). Recently, Mao and co-workers (2011) have performed substitutions in the prototype **29** in order to

Bioisosterism

Increase carbon chain using propyl and butyl. The compounds **30** was two times less active ($IC_{50} = 1.8 \mu M$) than **29** ($IC_{50} = 0.9 \mu M$), and **31** had similar activity ($IC_{50} = 0.9 \mu M$) when compared prototype (Figure 8) (Mao et al., 2011).

Bioisosterism term is applied in compounds which have similar physico-chemical and biological effects. This molecular modification is based on the substitution of molecular fragments, as for example, a functional group by another which similar physico-chemical properties (Thornber, 1957). Bioisosterism was classified into two categories: classic and non-classic. Classic bioisosterism is classified through valence atoms, chemical groups and aromatic rings (Table 1) (Patini and LaVoie, 1996; Barreiro and Fraga, 2008).

A classic example of drugs obtained from bioisosterism are fluoroquinolones. These compounds are known to inhibit bacterial DNA replication and transcription. Fluoroquinolones are used mainly in patients with multidrug-resistance tuberculosis (MDR-TB). The more usual fluoquinoles used in TB are: ciprofloxacin, sparfloxacin, ofloxacin, moxifloxacin and levofloxacin (Figure 9) (Renau et al., 1996).

Linezolid is an antibacterial agent used in the treatment of pneumonia caused by gram-negative bacteria. It was evaluated against MDR showing interesting result. So, some bioisosteres were developed, as PNU-100480, radezolid and torelozid (Figure 10) (Ford et al., 2001; Pinon et al., 2010; Leach et al., 2011).

New drugs antitubercular in clinical trial

Since the discovery of rifampicin in 60's none new drug was introduced in the market to treat TB. Currently, some drugs are under clinical trial by several public and private partnerships (Dover and Coxon 2011).

Nowadays, pharmaceutical partnerships have contributed to TB drug development. One example is phase II clinical trial of TMC207 and PA-824 (Figure 11). TMC207, a new ATP synthase inhibitor with wide antimycobacterium spectrum, is being evaluated in clinical trials. This diarylquinoline was first synthesized by Andries et al (2005) and showed high activity against MTB resistant to isoniazid and pyrazinamide with MIC = 0.01 and 0.03 $\mu g/mL$ respectively (Andries et al., 2005).

PA-824, a metronidazole analogue, was discovered

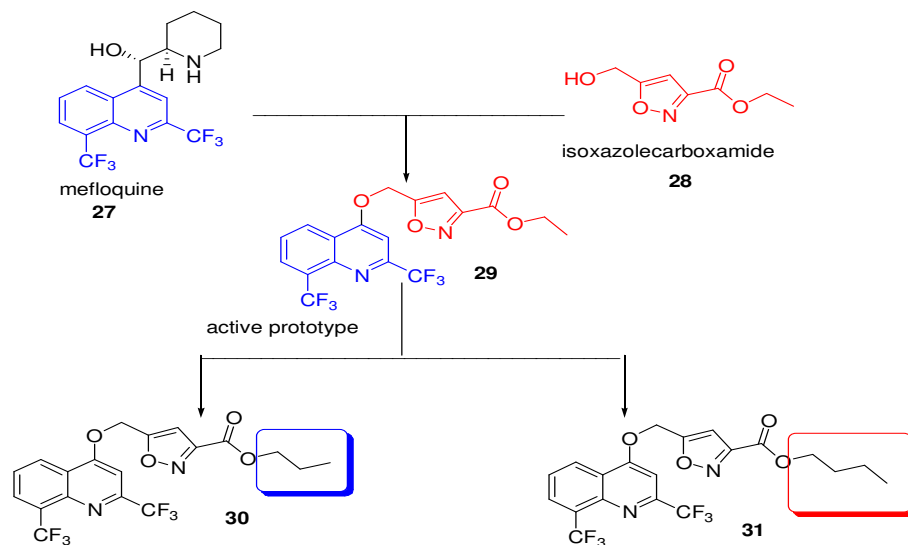











Figure 8. New prodrugs obtained by Mao and co-workers (2011).

Table 1. Bioisosteres classical group and atoms

Monovalent	Divalent	Trivalent	Tetravalent	Equivalence rings
F, OH, NH ₂ , CH ₃	-CH ₂ -	CH-	=C=	
Cl, SH ₂ , PH ₂ , Si ₃ , SR	-O-	=N-	=Si=	
Br	-S-	=P-	=N ⁺ =	
I	-Se-	=As-	=P ⁺ =	
	-Te-	=Sb-	=As ⁺ =	
			=Sb ⁺ =	
				
				
				

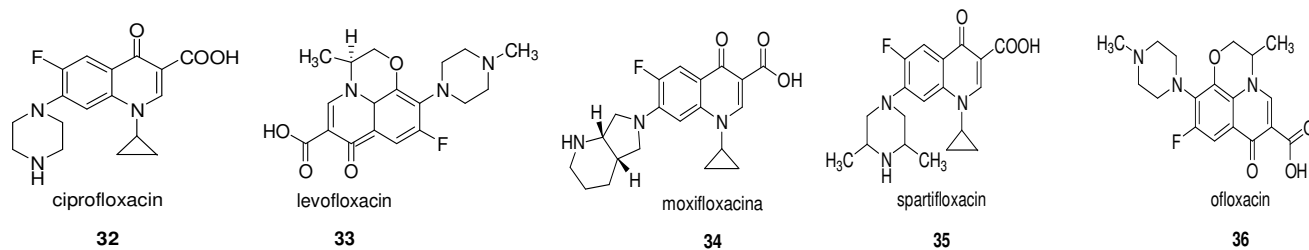


Figure 9. Fluoroquinolones obtained by bioisosterism.

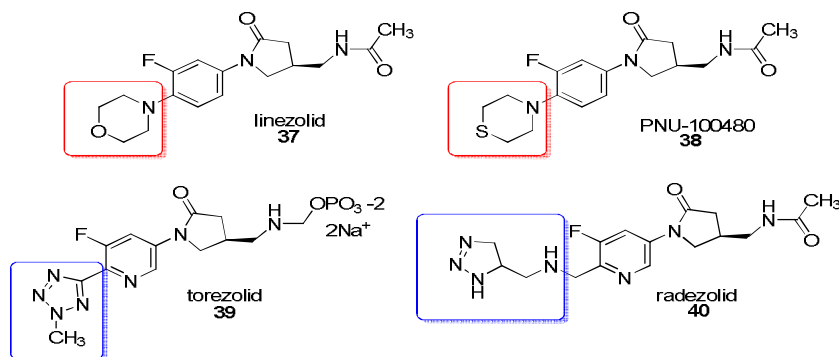


Figure 10. Chemical structure of linezolid derivatives by bioisosterism.

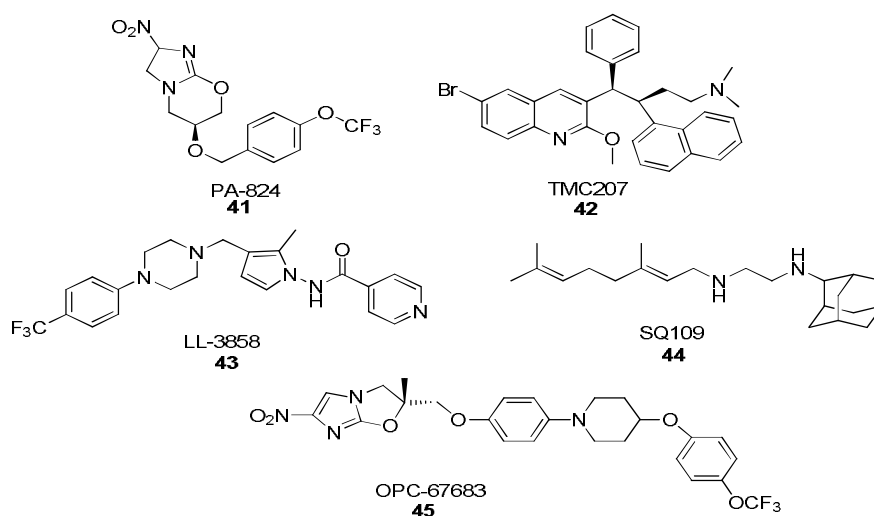


Figure 11. Antitubercular compounds currently in clinical trial.

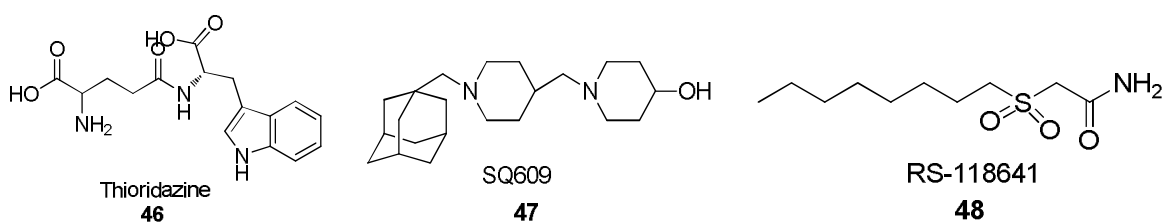


Figure 12. Promising compounds as antitubercular.

as chemotherapeutic agents (Angrawal et al., 1979) and currently is being evaluated in clinical trial as antitubercular drug. *In vitro* activity studies, PA-824 showed MIC value of 0.015 $\mu\text{g/ml}$ without cross-resistance with current drugs (Strover et al., 2000).

Compounds as LL-3858, ethambutol analogue SQ109 (cell wall multitarget inhibitor) and mitronidazol analogue OPC-67683 (cell wall multitarget inhibitor) also began their clinical trial recently by Lupin Ltd, Otsuka Pharmaceutica and Sequella Inc respectively

with interesting results (Figure11) (Dover and Coxon, 2011).

Thioridazine, initially known as neuroleptic, has showed to be a potent antitubercular providing cure inclusive in XDR-TB patients. The trial performed in India (Mumbai) and Argentina (Buenos Aires), demonstrated cure of 80% of the XDR-TB patients evaluated. Others two interesting drugs under clinical trial are SQ609 and RS-118641 (Figure 12) (Amaral et al., 2010; Dover and Coxon, 2011).

CONCLUSION

Nowadays TB drug discovery is a challenge. Absence of efficacy, resistance, poor compliance and comorbidities are some factors that difficult TB treatment. The molecular modification is an important and promising tool to discovery new antitubercular drugs, this fact can be perceived in the current pipeline TB drugs in clinical trial that almost of them were obtained using this strategy. Furthermore, many problems associated with efficacy, treatment time, drug resistance and adverse effects can be resolved using this strategy.

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REFERENCES

- Agrawal KC, Kathleen B. Bears, RKS (1979). Potential radiosensitizing agents. Dinitroimidazoles. *J. Med. Chem.* 22: 593–586.
- Amaral L, Boereeb MJ, Gillespie SH, Udwardi ZF, Soilingen Dv (2010). Thioridazine cures extensively drug-resistant tuberculosis (XDR-TB) and thence for global trials is now!. *Int. J. Antimicrob. Agents.* 35: 524–526.
- Andries K, Verhasselt P, Guillemont J, Goëhlmann HWH, Jean-Marc N, Winkler H, Gestel JV, Timmerman P, Zhu M, Lee E, Williams P, Chaffoy D, Huitric E, Hoffner S, Cambau E, Truffot-Pernot C, Lounis N, Jarlier V. A (2005). Diarylquinoline Drug Active on the ATP Synthase of *Mycobacterium tuberculosis*. *Science* 307: 223–227.
- Ansizu S, Moreno E, Solano B, Villar R, Buerquete A, Torres E, Pérez-Silanes S, Aladana L, Monge A (2010). New 3-methylquinoxaline-2-carboxamide 1,4-di-*N*-oxide derivatives as anti-*Mycobacterium tuberculosis* agents *Bioorg. Med. Chem.* 18: 2713–2719.
- Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, Collins D, de Lisle G, Jacobs WR (1994). *InhA*, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. *Science.* 263: 227–230.
- Barreiro, EJ; Fraga, CAM (2008). As bases moleculares da ação dos fármacos. *Química medicinal.* e. 2, 271–271.
- Cegielski JP, McMurray DN (2004). The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals. *Int. J. Tuberc. Lung Dis.* 8: 286–298.
- Chung WJ, Kornilov A, Brodsky BH, Higgins M, Sanchez T, Heifets L B, Cynamon M H, Welch J (2008). Inhibition of *M. tuberculosis* in vitro in monocytes and in mice by aminomethylene pyrazinamide analogs. *Tuberculosis.* 88: 410–419.
- Daniel TM, Bates JH, Downes KA, Bloom BR. (1994). History of Tuberculosis. In *Tuberculosis: Pathogenesis, Protection, and Control*. Ed. American Society for Microbiology: Washington, DC, USA, 13–24.
- De Rossi E, Aínsa JA, Riccardi G (2006). Role of mycobacterial efflux transporters in drug resistance: an unresolved question. *FEMS Microbiol Rev.* 30: 36–52.
- Dover LG, Coxon GD (2011). Current Status and Research Strategies in Tuberculosis Drug Development. *J. Med. Chem.* 54: 6157–6165.
- Finken M, Kirschner P, Meier A, Wrede A, Böttger EC (1993). Molecular basis of streptomycin resistance in *Mycobacterium tuberculosis*: alterations of the ribosomal protein S12 gene and point mutations within a functional 16S ribosomal RNA pseudoknot. *Mol. Microbiol.* 9: 1239–1246.
- Ford CWGE, Zurenko MR (2001). The discovery of linezolid, the first oxazolidinone antibacterial agent. *Curr. Drug Targets Infect. Disord.* 1: 181–199.
- Havilir DV, Barnes PF (1999). Tuberculosis in patients with human immunodeficiency virus infection. *New Engl. J. Med.* 340: 367–373.
- Haydel, SE (2010). Extensively Drug-Resistant Tuberculosis: A Sign of the Times and an Impetus for Antimicrobial Discovery. *Pharmaceuticals.* 3: 22683–2290.
- Imramovský A, Slovenko P, Vins J, Kocevar M, Jampilek J, Reckova Z, Kaustova J (2007). A new modification of anti-tubercular active molecules. *Bioorg. Med. Chem.* 15: 2551–2559. Trabulsi LR, Alterthum F (2005). *Microbiologia.* e. 4 409-413.
- Leach KL, Brickner SJ, Noe MC, Miller PF(2011). Linezolid, the first oxazolidinone antibacterial agent. *Ann. N.Y. Acad. Sci.* 1222: 49–54.
- Mao J, Wang Y, Wan B, Kozikowski AP, Franzblau SG (2007). Design, Synthesis, and Pharmacological Evaluation of Mefloquine-Based Ligands as Novel Antituberculosis Agents. *ChemMedChem.* 2: 1624–1630.
- Mao J, Yuan H, Wang Y, Wana B, Pak D, He R, Scott G (2010). Franzblau. Synthesis and antituberculosis activity of novel mefloquine-isoxazole carboxylic esters as prodrugs an Impetus for Antimicrobial Discovery. *Bioorg. Med. Chem. Lett.* 20: 1263–1268.
- Maus CE, Plikaytis BB, Shinnick TM (2005). Mutation of *tlyA* confers capreomycin resistance in *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* 49: 571–577.
- Patani GA, LaVoie E J (1996). Bioisosterism: A Rational Approach in Drug Design. *Chem. Rev.* 96: 3147–3176.
- Piddock LJ (2006). Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clin. Microbiol. Reviews.* 19: 382–402.
- Pinon M, Scoffaro C, Bignamini E, Cordola G, Esposito I, Milano R, Mignone F, Bertaina C, Tovo PA (2010). Two pediatric cases of multidrug-resistant tuberculosis treated with linezolid and moxifloxacin. *Pediatrics.* 126:1253–1256.
- Pitchenik AE, Fertel D, Bloch AB (1988). Mycobacterial disease: epidemiology, diagnosis, treatment, and prevention. *Clin. Chest Med.* 9: 425–441.
- Raviglione MC, Smith I (2007). XDR tuberculosis-implications for global public health. *New Engl. J. Med.* 356: 656–659.
- Renau TE, Sanchez JP, Gage JW, Dever JA, Shapiro MA, Gracheck SJ, Domagala JM (1996). Structure-activity relationship of the quinolone antibacterial against mycobacteria: effect of structural changes at N-1 and C-7. *J. Med. Chem.* 39: 729–735.
- Rengarajan J, Sassetti CM, Naroditskaya V. Sloutsky A, Bloom BR, Rubin EJ (2004). The folate pathway is a target for resistance to the drug para-aminosalicylic acid (PAS) in mycobacteria. *Mol. Microbiol.* 53: 275–282.
- Russell DG (2011). Mycobacterium tuberculosis and the intimate discourse of a chronic infection. *Immunol. Reviews.* 240: 252–268.
- Santos JL, Yamasaki PR, Chung MC, Takashi CH, Pavan FR, Leite CQF (2009). Synthesis and in vitro anti *Mycobacterium tuberculosis* activity of a series of phthalimide derivatives. *Bioorg. Med. Chem.* 17: 3795–3799.
- Scorpio A, Lindholm-Levy P, Heifets L, Gilman R, Siddiqi S, Cynamon M, Zhang Y (1997). Characterization of *pncA* mutations in pyrazinamide-resistant *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* 41: 540–543.
- Silva ATA, Castro L F, Guido RVC, Chung MC, Ferreira EI (2005). Advances in prodrug design. (Mini-Rev. *Med. Chem.* 5: 893–914.
- Simões MF, Valente E, Gómez MJ, Anes E, Constantino L (2009). Lipophilic pyrazinoic acid amide and ester prodrugs stability, activation and activity against *M. tuberculosis*. *Eur. J. Pharm. Sciences.* 37: 257–26.
- Sly LM, Suzanne M, Hingley-Wilson, Reiner NE, McMaster WR (2003). Survival of *Mycobacterium tuberculosis* in Host Macrophages Involves Resistance to Apoptosis Dependent upon Induction of Antiapoptotic Bcl-2 Family Member Mcl-11. *J. Immunol.* 170: 430–437.
- Smith NH, Hewinson RG, Kremer K, Brosch R, Gordon SV (2009). Myths and misconceptions: the origin and evolution of *Mycobacterium tuberculosis*. *Nature Reviews Microbiol.* 7: 537–544.

- Stover C K, Warrener P, VanDevanter DR, Sherman DR, Arain TM, Langhorne MH, Anderson SW, Towell JA, Yuan Y, McMurray DN, Kreiswirth BN, Barryk CE, Baker WR. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis (2000). *Nature*. 405: 962–966.
- Suzuki Y, Katsukawa C, Tamaru A, Abe C, Makino M, Mizuguchi Y, Taniguchi H (1998). Detection of kanamycin-resistant *Mycobacterium tuberculosis* by identifying mutations in the 16S rRNA gene. *J. Clin. Microbiol.* 36: 1220–1225.
- Takiff HE, Salazar L, Guerrero C, Philipp W, Huang WM, Kreiswirth B, Cole ST, Jacobs W.R, Telenti A (1994). Cloning and nucleotide sequence of *Mycobacterium tuberculosis gyrA* and *gyrB* genes and detection of quinolone resistance mutations. *Antimicrob. Agents Chemother.* 38: 773–780.
- Telenti A, Philipp WJ, Sreevatsan S, Bernasconi C, Stockbauer KE, Wieles B, Musser JM, Jacobs WRJr (1997). The *emb* operon, a gene cluster of *Mycobacterium tuberculosis* involved in resistance to ethambutol. *Nat. Med.* 3: 567–570.
- Thornber CW (1979). Isosterism and molecular modification in drug design. *Chem. Soc. Review.* 8: 563–580.
- Viegas-Junior C, Danuello A, Bolzani VS, Barreiro EJ, Fraga CA (2007). Molecular hybridization: a useful tool in the design of new drug prototypes. *Curr. Med. Chem.* 14: 1829–52.
- Williams DL, Spring L, Collins L, Miller LP, Heifets LB, Gangadharam PR, Gillia TP (1998). Contribution of *rpoB* mutations to development of rifamycin cross-resistance in *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* 42: 1853–1857.
- World Health Organization (2010). Global Tuberculosis Control.
- World Health Organization (2011). Global Tuberculosis Control.
- Zhang Y, Heym B, Allen B, Young D, Cole S (1992). The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. *Nature.* 358: 591–593.