ECRONICON OPEN ACCESS

EC PULMONOLOGY AND RESPIRATORY MEDICINE

Review Article

Natural Products a Reservoir of Drugs for Treatment of Pulmonary Tuberculosis

Grace Chitima Mugumbate*, Lorraine Yamurai Bishi and Freeborn Rwere

Department of Chemistry, School of Natural Sciences and Mathematics, Chinhoyi University of Technology, Chinhoyi, Zimbabwe

***Corresponding Author**: Grace Chitima Mugumbate, Department of Chemistry, School of Natural Sciences and Mathematics, Chinhoyi University of Technology, Chinhoyi, Zimbabwe. E-Mail: gmugumbate@cut.ac.zw

Received: June 21, 2018; **Published:** July 09, 2018

Abstract

Tuberculosis continues to cause health havoc worldwide, more than 90% of the reported cases are due to pulmonary tuberculosis. The treatment of tuberculosis was under control for decades; however this was challenged by the rapid emergence of multi-drug, extreme and recently total drug resistant strains of *Mycobacterium tuberculosis*, the causative agent of the disease. Therefore researchers are urgently hunting for new chemical entities that can be used in the development of novel tuberculosis drugs and also in identification of their targets. This review gives an overview of the current drugs used in pulmonary chemotherapy, current challenges, natural products as sources for novel anti-tuberculosis compounds, and the importance of the mode of action and the targets for the natural products. A focus on natural products would encourage researchers to revisit nature for the much needed novel drugs for treatment of pulmonary tuberculosis.

Keywords: *Natural Products; Pulmonary Tuberculosis; Drug Resistance; Targets*

Abbreviations

ATP: Adenosine Triphosphate; DOTS: Directly Observed Treatment Short-Course; DprE1- Decaprenylphosphoryl-D-Ribose 20 Epimerase; EMB- Ethambutol; FDA: Food and Drug Administration; INH: Isoniazid; MABA: Microplate Alamar Blue Assays; MDR: Multi-Drug Resistant; MIC: Minimum Inhibitory Concentration; *Mtb*: *Mycobacterium tuberculosis*; NP: Natural Products; PAS: Para-Aminosalicylate; pTB: Pulmonary Tuberculosis; PZA: Pyrazinamide; RIF: Rifampin; Tb: Tuberculosis; TDR: Total-Drug Resistant; WHO: World Health Organisation; XDR: Extensively-Drug Resistant

Introduction

Tuberculosis (TB) is a major global threat for humans, affecting about one third of the world's population, and in 2016 the disease caused about 1.4 million cases that resulted in ~ 500 000 deaths, 90% of which occurred in sub-Saharan Africa [1]. As a result the disease, caused by the bacterium *Mycobacterium tuberculosis (Mtb)*, poses massive negative effects on the health, economic and social status of people in mainly developing countries [2]. A particular problem associated with *Mtb* is that it can survive inside macrophages after phagocytosis, unless its cells are activated by cytokines produced by T-lymphocytes making the search for new effective treatment of TB challenging [3].

Amongst the various types of *Mtb* infections, lung infection responsible for pulmonary TB (pTB) constitutes more than 90% of all recorded TB cases. As a result, active pTB is responsible for most *Mtb* infections through exhalation of the causative agent, *tubercule bacilli*,

by patients suffering from the disease [2]. Treatment of pTB is mainly based on chemotherapy involving drugs used as first-line regimen for initial treatment and second line regimen for patients who have shown resistance to at least one of the first line drugs e.g. isoniazid and rifampicin [4]. Unfortunately, the high incidences of infection and increased emergence rates of multi-drug resistant (MDR), extensively-drug resistant (XDR) and recently total drug resistant (TDR) strains of the organism [5] and comorbidity with HIV and other opportunistic diseases continually complicates the TB control measures. Over the years, the quest for novel anti-pTB drugs has led to advancements in medicinal chemistry and combinatorial chemistry resulting in thousands of synthetic compounds released into the public domains, which serve as sources for new drug entities. However, these compounds lack individuality in the sense that new compounds are developed based on available chemical scaffolds. Historically, nature has been the source for new chemical entities used in the development of most antibiotics like streptomycin. Therefore, in this mini review will summarise current TB drugs, the different classes of new anti-TB natural products (NPs) with a focus on pulmonary tuberculosis, computational NP target identification used to facilitate identification of lead anti-TB NPs and their targets.

Treatment of Pulmonary TB

Current Status

Currently, TB chemotherapy is made up of a combination of first-line drugs, isoniazid, INH, rifampin, RIF administered for six months, pyrazinamide, PZA and ethambutol, EMB, given for two months (Figure 1). If the treatment fails as a result of bacterial drug resistance, or intolerance to one or more drugs, second-line drugs are used, such as para-aminosalicylate (PAS) and fluoroquinolones e.g. levofloxacin (Figure 2), aminoglycosides e.g. kanamycin, capreomycin, ethionamide and cycloserine, that are generally either less effective or more toxic with serious side effects [4]. To curb non-compliance, WHO introduced the Directly Observed Treatment Short-course (DOTS) program in 1995 [6] which consists of an initial phase of treatment with 4 drugs, INH, RIF, PZA and EMB, for 2 months daily, followed by treatment with INH and RIF for another 4 months, three times a week [7]. The drugs target various proteins in *Mtb* in that INH, inhibits synthesis of mycolic acid, a cell wall component (Garvin and Gorini, 2004), PZA targets the cell membrane whilst rifampin and streptomycin interferes with the initiation of RNA and protein synthesis respectively (Zhang., *et al*. 2000). In addition, EMB blocks biosynthesis of arabinogalactan, a major polysaccharide present in the mycobacterial cell wall [4], kanamycin and capreomycin, inhibit protein synthesis through modification of ribosomal structures at the 16S rRNA and cycloserine prevents the synthesis of peptidoglycan, a constituent of cell wall [4].

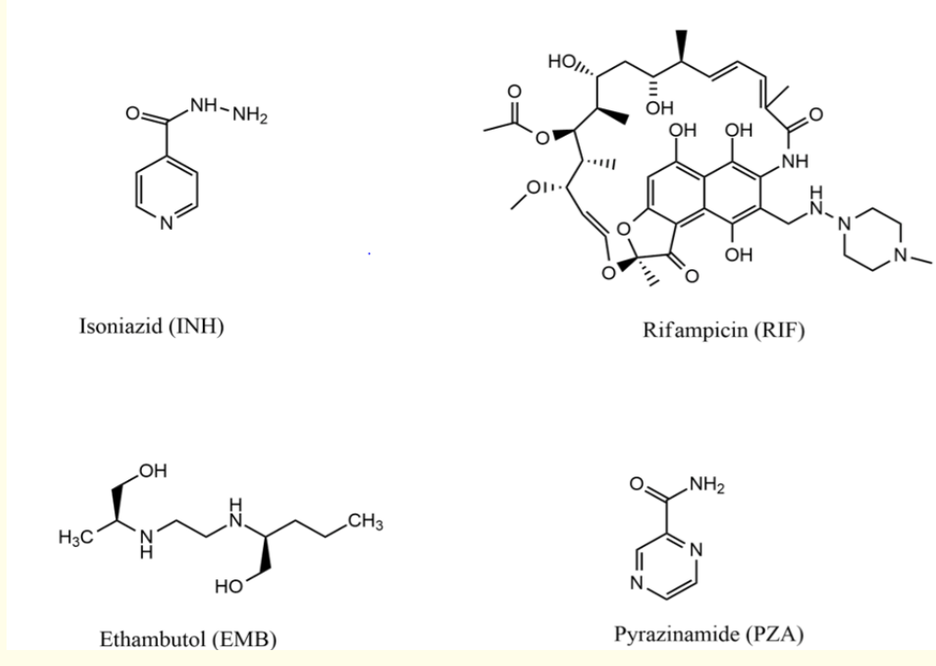

Figure 1: *Structures of first line anti-tuberculosis drugs.*

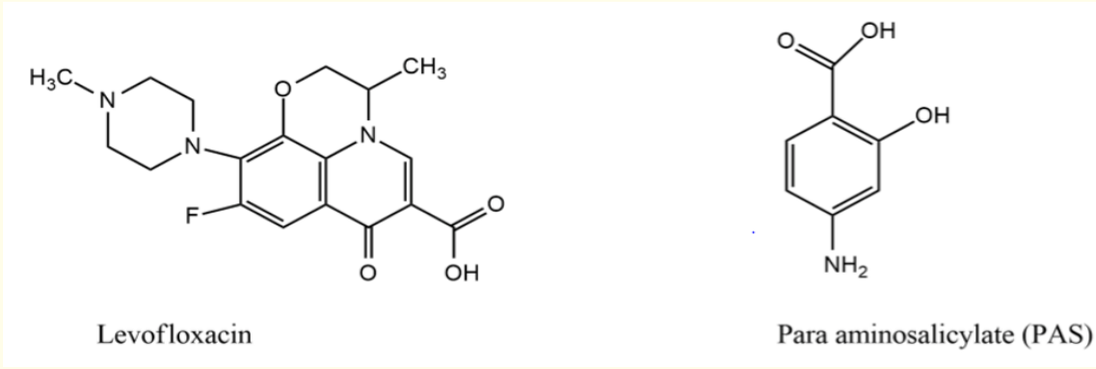

Figure 2: *Examples of second line TB drugs.*

In the present circumstances, due to MDR-TB and association between HIV and TB, DOTS is becoming rapidly fruitless in controlling tuberculosis. In 2008, WHO indicated that in areas where there are high incidences of MDR-TB, DOTS is failing to control the disease [6]. In such conditions, the second line drugs are prescribed in combination with DOTS drugs. However, this combination of drugs is very expensive, administering duration is long and has adverse side effects. The prolonged length of therapy makes patient compliance difficult, and such patients become potent source of drug-resistant strains. In addition, most of the TB drugs available today are ineffective against persistent *bacilli*, except for RIF and PZA. RIF is active against both actively growing and slow metabolizing non-growing *bacilli*, whereas PZA is active against semi-dormant non-growing *bacilli*. However, there are still persistent bacterial populations that are not killed by any of the available TB drugs [8].

New kids on the block

The rapid emergence of drug resistant pTB demands for rapid discovery of new anti-tuberculosis drugs, however it has taken about 4 decades for a new anti- tuberculosis drug to be released [9]. Fortunately, Mahajan R (2013), Deoghare S., *et al.* (2013) and Nagabushan H and Roopadevi HS (2014) reported the approval of Bedaquiline (Figure 3) by the US Food and Drug Administration (FDA) as the first anti-TB drug after the 40 years of dryness [9-11]. This new kid on the block is indicated for the treatment of multidrug-resistant tuberculosis (MDR-TB) in combination with at least three other antitubercular drugs when no other effective regimen is available [12] and it targets the bacterial adenosine triphosphate (ATP) synthase.

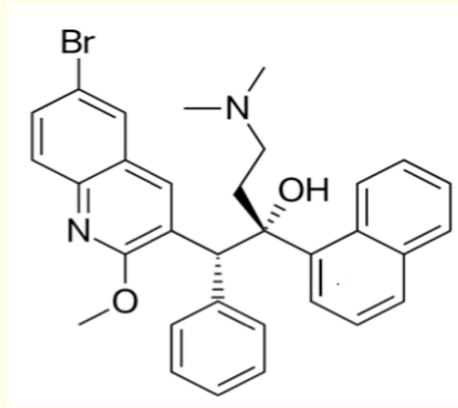

Figure 3: *Bedaquiline, the first anti-tuberculosis drug for treatment of multidrug resistant tuberculosis.*

Since then there has been a myriad of novel TB drugs either in preclinical trials or in phase I or II clinical trials. These include Delamanid (OPC-67683), another MDR-TB drug that contains the nitro-hydroimidazooxazole scaffold, and acts by inhibiting the synthesis of mycolic acid, displaying mode of action similar to that of isoniazid [13]. A number of authors have reported on these new drugs, a full list of old and new TB drugs and their modes of action can be found in the report by Kolyva AS and Karakousis PC [14].

Natural Products for pulmonary tuberculosis

Use of Plant Extracts

Plant-derived medicines have since been used in traditional medical systems for the treatment of numerous ailments worldwide (Sanusi., *et al*. 2017). Medicinal plants are a rich source of bioactive phytochemicals or bionutrients. Studies have shown that these phytochemicals play an important role in preventing many diseases like cancer, tuberculosis, diabetes and coronary heart disease [15]. The improved research interests in natural products in the hope of discovering new and novel antitubercular leads have been motivated by the increased incidences of MDR strains of *Mtb* and the adverse side effects associated with the first- and second-line antitubercular drugs [16]. In contrast, NPs have been, and will continue to be an abundant source of new drugs against many diseases because they are readily available and have less side effects [17]. The depth and breadth of therapeutic agents that have their origins in the secondary metabolites produced by living organisms cannot be compared with any other source of therapeutic agents [18]. Current advances have been made to speed up the discovery rate of novel TB drugs including diversifying strategies for environmental strains, high-throughput screening (HTS) assays, and chemical diversity [19].

In Zimbabwe, Chimponda and Mukanganyama in 2010 investigated thirty ethanol extracts from nineteen selected plants from Zimbabwe and screened them against *Mycobacterium aurum* and *Corynebacterium glutamicum* using the agar disk diffusion method. These two organisms were used as models for *Mycobacterium tuberculosis*. *Vernonia adoensis* and *Mangifera indica* extracts at 500 mg/disk had the highest growth inhibitory activity against *M. aurum* and *C. glutamicum* respectively. The extract from *Parinari curatellifolia* had an MIC of 8 µg/ disk and an MBC of 63 µg/disk; an MIC of 125 µg/disk and an MBC of > 500 µg/disk against *M. aurum* and *C. glutamicum* respectively. All the plant extracts were bacteriostatic and showed antagonistic effects when combined with rifampicin. They concluded that these plants might serve as a source of lead compounds in the search of new antimycobacterials with new mechanisms of action [20].

Additionally, several medicinal plants are traditionally used in Mozambique to treat tuberculosis and related symptoms. Luo., *et al.* in 2011 assessed the *in vitro* antimycobacterial activity of crude extracts from fifteen medicinal plants and revealed main classes of compounds which may account for the activity of extracts. The plant materials were sequentially extracted and broth micro-dilution method was employed to screen extracts against two mycobacterium species, *Mycobacterium smegmatis* ATCC 607 and *Mycobacterium tuberculosis* H37Rv. The extracts of *Maerua edulis* and *Securidaca longepedunculata*, *Tabernaemontana elegans* and *Zanthoxylum capense* were found to possess considerable activity against *Mycobacterium bovis BCG* and *Mycobacterium tuberculosis* H37Ra with MIC 15.6 - 62.5 µg/mL. Based on ^{1}H NMR spectroscopic analysis, they found major components (linear chain unsaturated fatty acids) in both *Maerua edulis* and *Securidaca longepedunculata* extracts. *Zanthoxylum capense* contained phenolic compounds and in *Tabernaemontana elegans* extracts, the indole alkaloids were prominent. The pronounced antimycobacterial activity of the medicinal plants *Maerua edulis, Securidaca longepedunculata, Zanthoxylum capense* and *Tabernaemontana elegans* suggested that they might provide compounds which could be potential anti-TB drug leads (Luo., *et al.* 2011).

Five Ethiopian medicinal plants, root *of Calpurnia aurea, seeds of Ocimum basilicum, leaves of Artemisia abyssinica, Croton macrostachyus* and *Eucalyptus camaldulensis* used locally for the management of TB were also evaluated against *M. tuberculosis* and *M. bovis* strains. The authors investigated for the *in vitro* antimycobacterial activity against *M. tuberculosis* and *M. bovis* strains. The crude 80% methanolic extracts of the root of *C. aurea*, seeds of *O. basilicum*, and leaves of *A. abyssinica, C. macrostachyus* and *E. camaldulensis* had anti-mycobacterial activity with minimum inhibitory concentration (MIC) ranging from 6.25 - 100 µg/mL. Their results supported the traditional use of these plants in the treatment of TB and hence their ability to be a reservoir for novel TB drugs. However, they concluded that further investigations were needed in isolating chemical constituents responsible for eliciting the observed activity in these plants [21].

Furthermore, Rajiniraja and Jayaraman, in 2014 investigated the antimycobacterial activity of selected medicinal plant extracts and screened phytochemicals responsible for mycobacterial inhibition. Serial extraction, antimicrobial assay and *Mycobacterium tuberculosis* assay were performed using the agar well diffusion method. They also determined the Minimum Inhibition Concentration by MABA assay and carried out qualitative phytochemicals test, contact bioautography and identification of active metabolites by spray reagents. Six medicinal plant extracts for antibacterial property using selected gram-positive and gram-negative organisms were identified. Activity guided serial extraction was performed to identify potential fractions responsible for antimicrobial activity, including the inhibition of *M. tuberculosis* H37Ra. Among the fourteen extracts prepared, plate assays indicated that petroleum ether and ethyl acetate extracts of *Alliums stadium* displayed substantial inhibition of *M. tuberculosis* growth. The study revealed the presence of a non–thio metabolite in *Allium sativum* with potential antimycobacterial activity [22].

In addition to the use of plant extracts, Gomez-cansino., *et al*. in 2017 stated that in Mexican Traditional Medicine, 187 plant species are used in the treatment of respiratory conditions that may be associated with tuberculosis. Among these, the most potent were *Aristolochia brevipes* (MIC = 12.5 µg/mL), followed by *Aristolochia taliscana*, *Citrus sinensis*, *Chrysactinia mexicana*, *Persea americana* and *Olea europaea* (MIC < 64 µg/ mL). Other potent extracts (inhibition > 95%, 50 µg/mL) include: *Amphipterygium adstringens*, *Larrea divaricata* and *Phoradendron robinsoni*. The most potent active compounds were identified from these plant species which include: Licarin A (isolated from *A. taliscana*), and 9-amino-9-methoxy-3,4-dihydro-2H-benzo[h]-chromen-2- one (transformation product of 9-methoxytariacuripyrone isolated from *Aristolochia brevipes*), both with MIC = 3.125 µg/mL, that is 8-fold less potent than the reference drug Rifampicin (MIC = 0.5 µg/mL). The natural compounds or extracts from plants undergo clinical trials or with animal models [23].

Jethva, Bhatt and Zaveri, 2017 studied nine plants that are thought to be medicinally important and evaluated their phytochemicals and anti-tubercular activity on *Mycobacterium smegmatis* using two different models. From the nine selected plants, three plants namely: *A. vasika, O. sanctum* and *A. galanga* showed maximum anti-tuberculosis activity at the MIC of 100 µg/ml, 250 µg/ml and 250 µg/ml respectively. Their study revealed the importance of plant extracts to control *Mycobacterial* infections which are being a threat to human health and for the development of alternate, safe and effective medicines [24].

Anti-tuberculosis Phytochemicals and their targets

Natural products continue to be the source for novel and diverse chemical entities for use in the development of alternative drugs [25]. In general active phytochemicals are isolated from plant extracts using a concerted effort of chromatographic techniques for separation in concert with a number of analytical techniques for compound identification. In their work, Mutai., *et al*. 2013 identified two new 3-hydroxyisoflavanones namely; kenusanone F 7-methyl ether, and sophoronol-7-methyl ether along with two known compounds (dalbergin and formononetin) that were isolated from the stem bark of *Dalbergia melanoxylon.* Their structures were elucidated by spectroscopic techniques. The authors reported that kenusanone F 7-methyl ether showed activity against *Mycobacterium tuberculosis*. From docking studies, kenusanone F 7-methyl ether and sophoronol-7-methyl ether displayed high binding affinity for *M. tuberculosis* drug target, INHA [26].

The *Mycobacterium tuberculosis* (*Mtb*) proteasome has been established as a viable target for the development of anti-tuberculosis agents. Zheng and co-workers in 2014 [27] analysed the inhibitory activities of 100 plant-derived natural products on the *Mtb* proteasome to identify novel potential inhibitors. Twelve of these natural products (10 of which were flavonoids) inhibited the activity of the *Mtb* proteasome by more than 65%. They compared structural differences between the flavonoids with good inhibitory activity and those without inhibitory activity and revealed that the hydroxyl at the flavonoid C ring C-3 or the hydroxyl/methoxyl at the flavonoid A ring C-6 were critical for the inhibition of proteasomal activity. They concluded that flavonoids represent a basis for rational structural design in the process of novel anti-tuberculosis drug discovery [7].

Compounds like benzothiazinones showed promising activity against mycobacterium, few compounds are in channel which may exhibit improved pharmacological effect. Decaprenylphosphoryl-D-ribose 20-epimerase (DprE1) is a flavoprotein which is a vulnerable target for antitubercular drug discovery, catalyses epimerization of decaprenylphosphoryl-D-ribose to decaprenylphosphoryl-D-arabinose through an intermediate formation of decaprenylphosphoryl-2-keto-ribose. This conversion makes DprE1 a potential drug target [3].

Physicochemical properties of natural products

Currently, the synthesis of oral drugs is guided by the Lipinski rule-of-five that summaries the optimum physicochemical properties required for bioavailability. These are molecular weight less than or equal to 500, logP and hydrogen bond donors less than or equal to 5, hydrogen bond acceptors less than or equal to 10 and number of rotatable bonds equal to 10 [28,29]. In light of this, Espinoza-Moraga., *et al*. 2013 investigated the difference between a diverse set of anti-*Mtb* natural products from plants and marine sources, and 25 TB drugs in current use or in development. The study revealed natural products and the TB drugs fall within the same physicochemistry space that the there was no significant difference in the extent of deviations from the rule-of-five [30].

Target identification for anti-tuberculosis natural products

The published genome of *Mtb* by Cole and co-workers in 1998, revealed more than 500 essential proteins in the pathogen, which provide a rich source for novel targets for new and current TB drugs [31]. However, more than 95% of these proteins are orphans since their ligands are still to be identified. In total, the first-line and the second-line drugs utilise just over 10 proteins during TB chemotherapy, reported in the ChEMBL database. The database also consists of thousands of anti-TB phenotypic hits deposited by researchers from industry and academia, whose targets are still to be identified [33]. Using experimental methods to match the targets to the ligands prove to be a backbreaking, time consuming and expensive process since it would involve expression and purification of about 500 proteins and performing the binding assays. However, computational methods that are fast and cost effective have taken a centre stage in de-orphaning *Mtb* targets using chemical and *Mtb* protein data. In previous work, we have used chemogenomic techniques that involves two ligand-based methods, machine learning and docking calculations to identify targets for natural products and phenotypic synthetic compounds. Firstly, using docking calculations the *Mtb* enzyme arabinosyltransferase EmbC was identified as a suitable target for the natural product scytoscalarol whilst the compound (-)-8-hydroxymanzamine binds to oxidoreductase *InhA* [28]. Furthermore, computational methods in conjunction with *in vitro* assays identified *Mtb* Dihydrofolate reductase as a target of about eight TB phenotypic hits [31]. The results from this work gave insights in the binding modes and binding interactions of the ligands (Figure 4) and the docking free energy of binding indicated that the compounds had high binding affinity with the target.

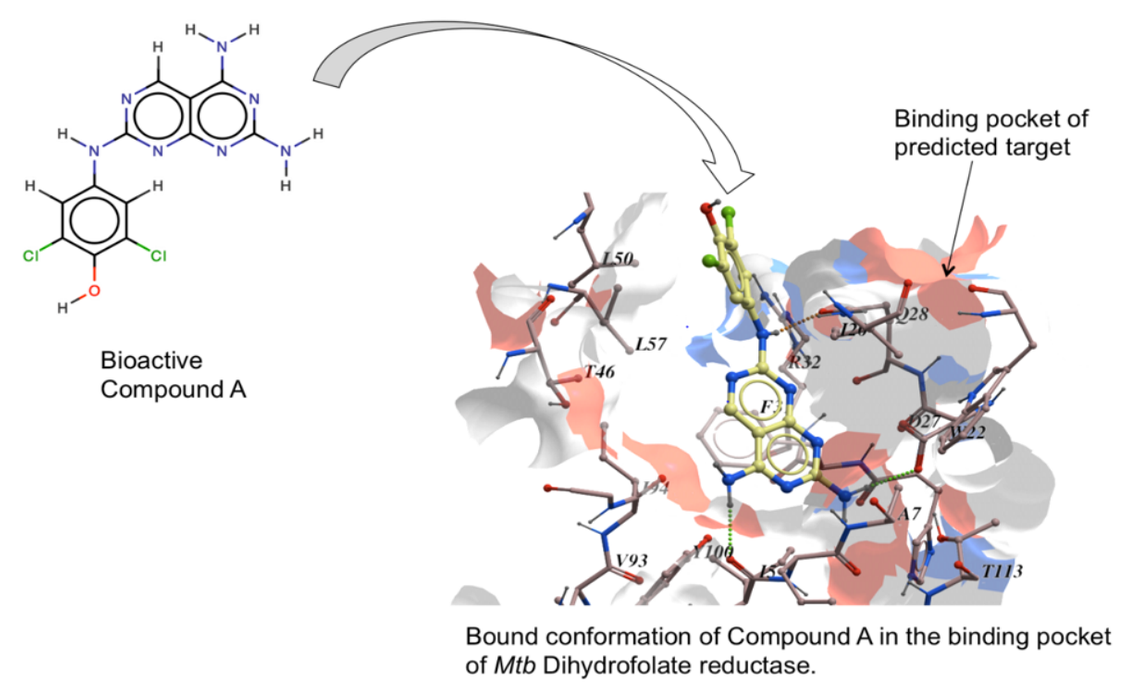

Figure 4: *Bioactive compound A bound to Dihydrofolate reductase indicating the binding orientation and the binding interactions.*

In follow up work, we identified ligands for HTH-transcriptional regulator, *EthR* using computational methods in conjunction with biophysical methods which lead to generation of eight crystal structures of the bound target-ligand complexes [34]. The information from these results are important for structure-based drug design of novel anti-pulmonary tuberculosis and pave way for enhanced target identification for NPs and the fight against the disease.

Conclusion

The future of pulmonary tuberculosis chemotherapy

The advancements in genomics have led to the release of the *Mycobacterium tuberculosis* genome and other pathogens which have a potential to infect people already infected with TB, opening new avenues for the discovery of novel anti-tuberculosis drugs. The introduction of genetic variations and personalised medicines is also bringing in a new dimension in the discovery of new effective and selective drugs and giving hope for novel pTB drugs. To compliment this, a number of natural product databases, for example AfroDB [35], have been published which provide easy access to anti-tuberculosis data and identification of new chemical entities for generation of drugs not prone to resistance. Furthermore', the development of high speed computers, appropriate softwares, target identification methods and new techniques that include machine learning and deep learning when applied effectively provide fast and cost effective pathways for the discovery of new TB drugs and hence contribute to elimination of the deadly disease.

Acknowledgements

The authors are grateful to Chinhoyi University of Technology for their support.

Bibliography

1. WHO. "Global Tuberculosis Report 2017". WHO (2017).
2. Delogu G., *et al*. "The biology of mycobacterium tuberculosis infection". *Mediterranean Journal of Hematology and Infectious Diseases* 5.1 (2013): e2013070.
3. Gawad J and Bonde C. "Current Affairs, Future Perspectives of Tuberculosis and Antitubercular Agents". *Indian Journal of Tuberculosis* 65.1 (2018): 15-22.
4. Zhang Y., *et al*. "New drug candidates and therapeutic targets for tuberculosis therapy". *Drug Discovery Today* 11.1-2 (2006): 21-27.
5. Sahu NU., *et al*. "Hit discovery of Mycobacterium tuberculosis inosine 50-monophosphate dehydrogenase, GuaB2, inhibitors". *Bioorganic and Medicinal Chemistry Letters* 128.10 (2018): 1714-1718.
6. World Health Organization. "Guidelines for the programmatic management of drug-resistant tuberculosis Guidelines for the programmatic management of drug-resistant tuberculosis". World Health Organization (2008): 12-185.
7. World Health Organization. "Global Tuberculosis Report 2017: Leave no one behind - Unite to end TB" (2017).
8. Zhang Y. "Persistent and dormant tubercle bacilli and latent tuberculosis". *Frontiers in Bioscience* 9 (2004): 1136-1156.
9. Deoghare S. "Bedaquiline: a new drug approved for treatment of multidrug-resistant tuberculosis". *Indian Journal of Pharmacology* 45.5 (2013): 536-537.
10. Mahajan R. "Bedaquiline: first FDA-approved tuberculosis drug in 40 years". *International Journal of Applied and Basic Medical Research* 3.1 (2013): 1-2.
11. Nagabushan H and Roopadevi HS. "Bedaquiline: a novel antitubercular drug for multidrug-resistant tuberculosis". *Journal of Postgraduate Medicine* 60.3 (2014): 300-302.

12. Worley MV and Estrada SJ. "Bedaquiline: a novel antitubercular agent for the treatment of multidrug-resistant tuberculosis". *Pharmacotherapy* 34.11 (2014): 1187-1197.

13. Gler MT., *et al*. "Delamanid for multidrug-resistant pulmonary tuberculosis". *New England Journal of Medicine* 366.23 (2012): 2151-2160.

14. Kolyva AS and Karakousis PC. "Old and new TB drugs: mechanisms of action and resistance". In Understanding Tuberculosis-New Approaches to Fighting Against Drug Resistance. InTech (2012).

15. Kurmukov AG. "Phytochemistry of medicinal plants". Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan 1.6 (2013): 13-14.

16. Hoffner S. "Multidrug-resistant tuberculosis: The problem and some priorities in controlling it". *International Journal of Mycobacteriology* 5.1 (2016): S59.

17. Salomon CE and Schmidt LE. "Natural Products as Leads for Tuberculosis Drug Development". *Current Topics in Medicinal Chemistry* 12.7 (2012): 735-765.

18. Nguta JM., *et al*. "Current perspectives in drug discovery against tuberculosis from natural products". *International Journal of Mycobacteriology* 4.3 (2015): 165-183.

19. Liu X., *et al*. "Exploring anti-TB leads from natural products library originated from marine microbes and medicinal plants". *Antonie Van Leeuwenhoek* 102.3 (2012): 447-461.

20. Chimponda T and Mukanganyama S. "Antimycobacterial activities of selected medicinal plants from Zimbabwe against Mycobacterium aurum and Corynebacterium glutamicum". *Tropical Biomedicine* 27.3 (2010): 595-610.

21. Gemechu A., *et al*. "In vitro anti-mycobacterial activity of selected medicinal plants against Mycobacterium tuberculosis and Mycobacterium bovis strains". *BMC Complementary and Alternative Medicine* 13 (2013): 291.

22. Rajiniraja M and Jayaraman G. "Bioautography guided screening of selected indian medicinal plants reveals potent antimycobacterial activity of allium sativum extracts- implication of non sulfur compounds in inhibition". *International Journal of Pharmacy and Pharmaceutical Sciences* 6.5 (2014): 671-676.

23. Gómez-cansino R., *et al*. "Natural Compounds from Mexican Medicinal Plants as Potential Drug Leads for Anti-Tuberculosis Drugs". *Anais da Academia Brasileira de Ciências* 89.1 (2017): 31-43.

24. Jethva K., *et al*. "Phytochemical evaluation and anti-tuberculosis activity of selected nine ethnomedicinal plants". *International Journal of Herbal Medicine* 5.1 (2017): 25-30.

25. Sánchez JGB and Kouznetsov VV. "Antimycobacterial susceptibility testing methods for natural products research". *Brazilian Journal of Microbiology* 41.2 (2010): 270-277.

26. Mutai P., *et al*. "3-Hydroxyisoflavanones from the stem bark of Dalbergia melanoxylon: Isolation, antimycobacterial evaluation and molecular docking studies". *Phytochemistry Letters* 6.4 (2013): 671-675.

27. Zheng Y., *et al*. "Identification of plant-derived natural products as potential inhibitors of the Mycobacterium tuberculosis proteasome". *BMC Complementary and Alternative Medicine* 14 (2014): 400.

28. Lipinski CA. "Lead-and drug-like compounds: the rule-of-five revolution". *Drug Discovery Today: Technologies* 1.4 (2004): 337-341.

29. Lipinski CA. "Drug-like properties and the causes of poor solubility and poor permeability". *Journal of Pharmacological and Toxicological Methods* 44.1 (2000): 235-249.

30. Espinoza-Moraga M., *et al*. "In silico comparison of antimycobacterial natural products with known antituberculosis drugs". *Journal of Chemical Information and Modeling* 53.3 (2013): 649-660.

31. Cole S., *et al*. "Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence". *Nature* 393.6685 (1998): 537-544.

32. Gaulton A., *et al*. "ChEMBL: a large-scale bioactivity database for drug discovery". *Nucleic Acids Research* 40.D1 (2011): D1100-D1107.

33. Mugumbate G., *et al*. "Mycobacterial dihydrofolate reductase inhibitors identified using chemogenomic methods and in vitro validation". *PloS one* 10.3 (2015): e0121492.

34. Mugumbate G., *et al*. "Target Identification of Mycobacterium tuberculosis Phenotypic Hits Using a Concerted Chemogenomic, Biophysical, and Structural Approach". *Frontiers in Pharmacology* 8 (2017): 681.

35. Ntie-Kang F., *et al*. "AfroDb: a select highly potent and diverse natural product library from African medicinal plants". *PLoS One* 8.10 (2013): e78085.

Volume 7 Issue 8 August 2018