Evaluation of anti-mycobacterial activity of a set of 250 chemical compound library

[a]Menikpurage, M., [a]Bassin, J.P., [b]Cox, J.A.G., [c]Reddy, T. R.K. and [a]Goyal, M.

[a]School of Life and Medical Sciences, University of Hertfordshire, Hatfield, Herts AL10 9AB, UK. [b]School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham B4 7ET, UK. [c]The Medicines Research Group, School of Health, Sport and Biosciences, University of East London, London E15 4LZ, UK.

Abstract

Recent advancement of high through-put screening of natural and synthetic chemical libraries to identify hit compounds with anti-TB properties has been highly useful in TB drug discovery. In this study 250 chemical compounds designed using InhA crystal structure were screened against three Mycobacterial species *M.aurum* ,*M.bovis* BCG and *M.abscessus* to evaluate their anti-mycobacterial activities using microbroth dilution method. Twenty-four compounds showed Minimum Inhibitory Concentrations (MIC) in the range of 16-64 against *M. aurum* whereas six compounds out of these showed MIC value of 32 $\mu g.ml^{-1}$ and one compound had a MIC of 16 $\mu g.ml^{-1}$. Ten compounds showed activity at MIC of 64 $\mu g.ml^{-1}$ against *M.bovis* BCG. Further, three compounds showed activity against *M.abscessus* at 256 $\mu g.ml^{-1}$. To improve the anti-mycobacterial activity of the most active compounds will be chemically modified for better anti-TB drug candidates.

Introduction

Drug discovery research on tuberculosis and non-tuberculosis mycobacterical species has a great important due to increased resistance against currently existing drugs. Tuberculosis (TB) is a pulmonary infection caused by *Mycobacterium tuberculosis* (M.tb). It infects at least one third of the world population and accounts for two million deaths each year (Libardo et al., 2018). Emergence of multi drug resistant (MDR) and extensively drug resistant (XDR) TB have resulted in massive treatment failures and high mortality rates. *M.abscessus* is a non-tubercular mycobacterium which mainly cause pulmonary lung disease, skin and soft tissue infections. Due to high drug resistance attained by the bacterium, infection rates and related complications are at a rise (Lee et al., 2015). Urgency for new anti-TB and non-TB drugs with new mechanisms of action are therefore crucial (Nayyar & Jain. 2005). Two main approaches are in place in drug discovery; target-to-drug and drug-to-target based drug discovery where the second approach involves whole cell screening of compound libraries to find the target/targets (Abraham & Besra, 2011).

The aim of the study was to screen a library of 250 synthetic compounds designed targeting InhA crystal structure for their *in vitro* anti-mycobacterial activity against three mycobacterium species *M.aurum , M.bovis* BCG and *M.abscessus.*

Methodology and results

A library of commercially synthesized 250 chemical compounds designed targeting Mtb InhA were purchased from Asinex Pharma and tested for their anti-mycobacterial activity against *M.aurum , M.bovis* BCG and *M.abscessus* using micro-broth dilution method. Serial dilutions of synthetic compounds and the positive controls, Isoniazid (INH) and Ciprofloxacin were made in triplicates in a 96 well plate. Following the incubation at 37 ^{0}C for two days (*M.aurum* and *M.abscessus*) and 7 days (*M.bovis* BCG) with microbial cultures, resazurin sodium salt (0.02%, 35 µl) was added in-order to determine MIC values of the compounds. The lowest concentration where the color change (blue to pink) occurred was taken as MIC for each compound.

As shown in Table 1, twenty-four compounds showed MIC values in the range of 16-64 $\mu g.ml^{-1}$ against *M. aurum.* Six of these compounds (BAS 16353479, BAS 5338721

,BAS 24803511, BAS 10396682, BAS 3395674 & BAS 4849985) showed MIC value at 32 $\mu g.ml^{-1}$. Three out of ten compounds which showed activity at 64 $\mu g.ml^{-1}$ against *M.bovis* BCG were also active against *M.aurum* at 32 $\mu g.ml^{-1}$ (BAS 16353479, BAS 16353479 and BAS 3395674). BAS 4323847 (Fig1) was the most active compound with the lowest MIC of 16 $\mu g.ml^{-1}$.

Three of the other compounds (BAS 8209246, ASN 9172075 and BAS 1251634) showed activity against *M.abscessus* at 256 $\mu g.ml^{-1}$.

Table 1: Active compounds, their MIC values against *M.aurum , M.bovis* BCG and *M.abscessus* and selective indices against murine J774 macrophage cell line.

Index	MIC with *M.aurum* (µg/ml)	MIC with *M.bovis* BCG (µg/ml)	MIC with *M.abscessus* (µg/ml)	Cytotoxicity and selective index
BAS 8303238	64	>128	>256	
BAS 728231	64	64	>256	
BAS 16353479	32	64	>256	36, 0.88
BAS 134197	64	>128	>256	
BAS 5601501	64	128	>256	
BAS 1841827	64	>64	>256	
BAS 8196551	64	>64	>256	
BAS 1884677	64	>64	>256	
BAS 9528815	64	>64	>256	
ASN 3430546	64	64	>256	
BAS 619014	64	>64	>256	
BAS 5338721	32	>64	>256	
BAS 11101623	64	>64	>256	
BAS 11101671	64	>64	>256	
ASN 13158981	64	64	>256	
BAS 24803511	32	64	>256	23, 1.39
ASN 10396682	32	>64	>256	
BAS 3395674	32	64	>256	8.5, 3.76
BAS 501131	64	>64	>256	
BAS 93464	>128	64	>256	
BAS 4849985	32	64	>256	29.75, 1.07
BAS 1218639	128	64	>256	
ASN 7441543	64	64	>256	
BAS 4323847	16	>64	>256	6, 2.66
BAS 1175958	64	>64	>256	
BAS 1906687	64	>64	>256	
BAS 8209246	>256	>256	256	132,0.5
ASN 9172075	>256	>256	256	132,0.5
BAS 1251634	>256	>256	256	36, 0.125
Isoniazid	1	1	N/A	22.5, 22.5
Ciprofloxacin	N/A	N/A	1	9,4.5

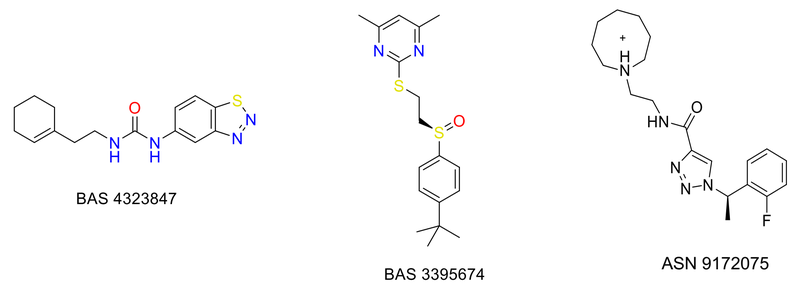

Figure 1: Chemical structures of BAS 4323847 which shows the lowest MIC of 16 $\mu g.ml^{-1}$ with *M.aurum* and BAS 3395674 which is active at 32 $\mu g.ml^{-1}$ & 64 $\mu g.ml^{-1}$ with *M.bovis* BCG. ASN 9172075 active against *M.abscessus* at 256 $\mu g.ml^{-1}$

Conclusion

According to the results obtained, few compounds have shown promising anti-mycobacterial activity. BAS 4323847, a urea derivative can be taken as the most active compound with the lowest MIC (16 $\mu g.ml^{-1}$). Urea substitutes are well documented for their anti-mycobacterial activity and in few recent studies, urea-based compounds have been tested against *M.tb* and shown to be potential anti-tb drug candidates(Konduri et al.,2021) & (Upadhyaya et al., 2009). Also, a study carried out by Brown et al.,(2011) suggested that epoxidase hydrolases as the probable target of urea derivatives tested in *M.tuberculosis*.

The functional groups of one of the active compounds, ASN 9122075 (Fig1) which showed activity against *M.abscessus* at 256 $\mu g.ml^{-1}$ are carboxamide, triazole group and a phenyl containing a fluorine atom. Drugs containing carboxamide groups are known to target the trehalose monomycolate transporter (MmpL3) which distrusts the mycolic acid synthesis in mycobacterial cell wall (Dupont et al., 2016).

Selective index >1 can be considered as non-toxic for murine J774 cells(Indrayanto, Putra & Suhud.,2021). Thus, further structural improvements will be required for compounds with SI <1to consider them in further research.

Future work

- These compounds will be tested against *Mtb* and *M.abscessus* clinical isolates.
- Active compounds will be subjected to structural changes for improved activity.

References

Abrahams. K.A. & Besra. G.S.(2011). Mycobacterial Drug Discovery. *Med.Chem. 11.1354*

Brown, J.R., North, J.N., Julian G., Morisseau,H.C., Jerrod S. & Sun, D.S.(2011) The Structure Activity Relationship of Urea Derivatives as Anti-Tuberculosis Agents. *Bioorg Med Chem.*

Dupont, C., Viljoen, A., Dubar, F., Blaise, M., Bernut, A., & Pawlik, A. et al. (2016). A new piperidinol derivative targeting mycolic acid transport in Mycobacterium abscessus. Molecular Microbiology, 101(3), 515-529. doi: 10.1111/mmi.13406

Indrayanto, G., Putra, G., & Suhud, F. (2021). Validation of in-vitro bioassay methods: Application in herbal drug research. Profiles Of Drug Substances, Excipients And Related Methodology, 273-307.

Konuri, S., Bhargavi, D., Prashanth, J., Krishna, V.S. & Rao, K.P.(2021).Design and synthesis of "Chloropicolinate Amides and Urea Derivatives" as Novel Inhibitors for Mycobacterium tuberculosis. *ACS Omega*,6,1657-1667.

Lee, M., Sheng, W. H., Hung, C. C., Yu, C. J., Lee, L. N., & Hsueh, P. R. (2015). Mycobacterium abscessus Complex Infections in Humans. Emerging infectious diseases, 21(9), 1638–1646.

Libardo, M.D.J., Boshoff, H.I.M. & Barry,C.E.(2018).The present state of the tuberculosis drug development pipeline. *Current opinion in Pharmacology.*42:81-94

Nayyar, A. & Jain, R. (2005). Recent advances in new structural classes of anti-tuberculosis agents. *Current medicinal chemistry.*12.1873-1886

Upadhayaya, R.S., Kulkarni, G.M., Vasireddy, N.R. Vandavasi, J.K ., Dixit, S.S. Sharma,V & Chattopadhyaya,J. (2009) Design, synthesis and biological evaluation of novel triazole, urea and thiourea derivatives of quinoline against Mycobacterium tuberculosis. *Bioorg. Med. Chem.* 17 (2009) 4681–4692