Qualitative anti-tubercular activity of synthetic ethyl 7-acetyl-2-substituted-3-(4-substituted benzoyl) indolizine-1-carboxylate analogues

Sandeep Chandrashekharappa1*, Katharigatta N. Venugopala2,3, Rashmi Venugopala4, Basavaraj Padmashali1,5*

1Department of Chemistry, Sahyadri Science College (Autonomous), Kuvempu University, Shimoga, India.
2Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Ahsa, Kingdom of Saudi Arabia.
3Department of Biotechnology and Food Technology, Faculty of Applied Science, Durban University of Technology, Durban, South Africa.
4Department of Public Health Medicine, University of KwaZulu-Natal, Howard College Campus, Durban, South Africa.
5Department of Studies and Research in Chemistry, School of Basic Sciences, Rani Channamma University, Belagavi, India.

ARTICLE INFO
Received on: 07/11/2018
Accepted on: 04/01/2019
Available online: 28/02/2019

Key words: Indolizine analogs, qualitative screening, agar dilution method, whole cell anti-tubercular screening, H37Rv strain, Mycobacterium tuberculosis.

ABSTRACT
Both the emergence of multidrug-resistant and extensively drug-resistant tuberculosis (TB) are currently the major challenges in the treatment of TB. Only delamanid and bedaquiline have been recently approved as anti-TB drugs in the past 40 years. In an attempt to search for active anti-TB compounds against the sensitive strain of Mycobacterium tuberculosis, H37Rv—a series of synthetic ethyl 7-acetyl-2-substituted-3-(4-substituted benzoyl)indolizine-1-carboxylates (2a–r)—have been screened for in vitro qualitative anti-TB activity using an agar dilution method. It was found that compounds 2a, 2b, 2c, 2f, 2g, 2i, 2j, 2l, 2o, 2p, and 2r, which have various functional groups on the indolizine nucleus, were active against the H37Rv strain.

INTRODUCTION
Tuberculosis (TB) is a chronic, infectious disease caused by Mycobacterium tuberculosis that has been present for a long time. This disease remains the most large-scale medical and social problem. Approximately 3–4 million people around the world die each year from TB, and every year, approximately 8 million first-ever events of TB are registered. This burden is due to the high susceptibility of human immunodeficiency virus-infected patients (Nunn et al., 2005). The emergence of multidrug-resistant and extensively drug-resistant TB has directed attention to, and scientific interest in, this infectious disease (Bloch et al., 1994; Kochi et al., 1993; Rastogi et al., 1992). For this reason, there is a need to discover new classes of chemical agents that features the diverse mechanisms of action to treat this disease.

Nitrogen-containing heterocyclic compounds have drawn the attention of medicinal chemists due to their various therapeutic properties (Nagesh et al., 2014; Siddesh et al., 2014; Threvini et al., 2014). Indolizines are bicyclic heteroaromatic compounds containing six- and five-membered condensed rings with bridging nitrogen (Sandee et al., 2013). Indolizine pharmacophore, with different degrees of substitution and unsaturation, is present in a wide variety of natural alkaloids (Michael, 2001; 2002) and unnatural azacyclic compounds (Halab et al., 2002; Pearson and Hembre, 1996). Synthetic indolizine analogs have been reported for their numerous pharmacological...
properties, such as their analgesic (Vaught et al., 1990), anti-cancer (Butler, 2008; Sandeep et al., 2016a), anti-diabetic (Mederski et al., 2012; January 31), anti-histaminic (Cingolani et al., 1990), anti-inflammatory (Hagishita et al., 1996; Sandeep et al., 2017; 2018b), anti-leishmanial (Jaisankar et al., 2004), anti-microbial (Hazra et al., 2011), anti-mutagenic (Olejnikova et al., 2009), antioxidant (Nasir et al., 1998), anti-tubercular (Dannhardt et al., 1987; Khedr et al., 2018), antiviral (Mishra and Tiwari, 2011), larvicidal (Sandeep et al., 2016b; 2018a), in vitro, and herbicidal activities (Smith et al., 2005).

With these observations in mind, and in continuation of our efforts to develop novel heterocyclic (Khedr et al., 2018; Venugopala et al., 2013; 2016; 2018) and peptide (Narayanaswamy et al., 2011) compounds with anti-TB activity and to screen pharmacologically active heterocyclic compounds based on their polymorphic properties (Munshi et al., 2004,

\[ \begin{align*}
\text{Reagents and conditions: (i) 4-substituted phenacyl bromide, acetone, 5 hours stir; (ii) K}_2\text{CO}_3, \text{dimethylformamide (DMF), 30 minutes stir at room temperature.}
\end{align*} \]
Table 1. Anti-tubercular activity against H37Rv strain of M. tuberculosis.

<table>
<thead>
<tr>
<th>Compound code</th>
<th>Concentration (µg/ml)</th>
<th>cLogP</th>
<th>25</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>1.5990</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2b</td>
<td>2.1814</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2c</td>
<td>2.8312</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2d</td>
<td>4.4772</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>2e</td>
<td>5.0062</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>2f</td>
<td>3.4512</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2g</td>
<td>3.4012</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2h</td>
<td>5.0472</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>2i</td>
<td>4.0212</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2j</td>
<td>3.5512</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2k</td>
<td>5.1972</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>2l</td>
<td>4.1712</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2m</td>
<td>3.8200</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>2n</td>
<td>4.3490</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>2o</td>
<td>2.7843</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2p</td>
<td>3.1840</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2q</td>
<td>4.8300</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>2r</td>
<td>3.8061</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Streptomycin</td>
<td></td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td></td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

Note: “cLogP” was calculated using the software package ChemBioDraw Ultra 13.0v (PerkinElmer Inc., Waltham, MA).

R indicates resistivity and S indicates the sensitivity of the tested compounds against the standard in different concentrations.

Panini et al., 2014; 2016b), we evaluated synthesized (Sandeep et al., 2016) ethyl 7-acetyl-2-substituted-3-(4-substituted benzoyl)indolizine-1-carboxylate analogs 2a–r (Scheme 1; Fig. 1) to determine their qualitative anti-TB activity in vitro using an agar dilution method against a susceptible H37Rv strain (Table 1).

MATERIALS AND METHODS

The synthetic route for the construction of indolizine scaffolds 2a–r and the characterization of the title compounds are described in our earlier research publication (Sandeep et al., 2016). The synthetic ethyl 7-acetyl-2-substituted-3-(4-substituted benzoyl)indolizine-1-carboxylate 2a–r have been tested for their qualitative anti-TB activity in vitro against an M. tuberculosis H37Rv strain.

Anti-tubercular activity

The procedure followed for anti-TB screening involves the use of Middlebrook 7H9 broth and the M. tuberculosis strain H37Rv. The basal medium was prepared in accordance with the manufacturer’s instructions (HiMedia Laboratories, Mumbai, India) and sterilized by autoclaving; 4.5 ml of broth was added into each sterile bottle. To this, 0.5 ml of Oleic acid dextrose catalase (OADC) supplement was added, which contained catalase, bovine serum albumin, and dextrose fraction. A volume of 10 mg/ml stock solution of the test compound was then prepared. From this, an appropriate amount of solution was transferred to media bottles to achieve concentrations of 25, 50, and 100 µg/ml. Then, 10 µl of a suspension containing the M. tuberculosis H37Rv strain (100,000 organisms/ml, adjusted by McFarland’s turbidity standard) was transferred to each tube and incubated at 37°C. In addition, one growth control without the compound and drug controls was established. The bottles were examined twice a week to determine growth, for a total period of 3 weeks. Turbidity was considered as growth and was indicative of resistance to the compound. Growth was confirmed by taking a smear from each bottle and conducting a Ziehl–Neelsen (ZN) stain. The antibiotic standards used included streptomycin (7.5 µg/ml) and pyrazinamide (7.5 µg/ml).

RESULTS AND DISCUSSION

The in vitro qualitative anti-TB activity was tested for all of the 2a–r derivatives using the M. tuberculosis strain H37Rv at 25, 50, and 100 µg/ml by an agar dilution method (Sun et al., 2010) (Table 1). Test compounds 2a, 2b, 2c, 2f, 2g, 2i, 2j, 2l, 2o, 2p, and 2r were found to be active against M. tuberculosis at all three concentrations. Compounds 2d, 2e, 2h, 2k, 2m, 2n, and 2p were found to be inactive against M. tuberculosis at all three concentrations. The common functionality of inactive compounds 2d, 2e, 2h, 2k, 2m, 2n, and 2p was the presence of a diethyl ester group at position 1 and a methyl or ethyl group at position 2. The common functionality of active compounds 2a, 2b, 2c, 2f, 2g, 2i, 2j, 2l, 2o, 2p, and 2r was the presence of a diethyl ester group at position 1, and either hydrogen or diethyl ester at position 2. The acetyl group was found at position 7 and the substituted benzoyl group was noted at position 3 of the indolizine nucleus.

CONCLUSION

In an attempt to select promising indolizine compounds (2a–r) to determine their quantitative anti-TB activity, test compounds 2a, 2b, 2c, 2f, 2g, 2i, 2j, 2l, 2o, 2p, and 2r were active against the M. tuberculosis H37Rv strain, while test compounds 2d, 2e, 2h, 2k, 2m, 2n, and 2p were found to be inactive against the M. tuberculosis H37Rv strain. Based on the preliminary results, we proposed to design and synthesize novel indolizine scaffolds having various functional groups for anti-TB activity against multidrug-resistant strains of M. tuberculosis.

ACKNOWLEDGMENTS

The authors would like to thank Sahyadri Science College, Shimoga, for providing laboratory facilities and Maratha Mandal NGS Institute of Dental Science, Belgaum, India for carrying out anti-tubercular activity. KNV would like to thank the National Research Foundation (Grant Nos. 96807 and 98884), South Africa and Durban University of Technology, South Africa, for their support and encouragement.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES


methyl, substituted phenyl-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylates against *Mycobacterium tuberculosis*. Drug Des Develop Ther, 2016; 10:2681.


**How to cite this article:** Chandrashekharappa S, Venugopala KN, Venugopala R, Padmashali B. Qualitative anti-tubercular activity of synthetic ethyl 7-acetyl-2-substituted-3-(4-substituted benzoyl) indolizine-1-carboxylate analogs. J Appl Pharm Sci, 2019; 9(02):124–128.