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

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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; Thriveni *et al*., 2014). Indolizines are bicyclic heteroaromatic compounds containing six- and five-membered condensed rings with bridging nitrogen (Sandeep *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

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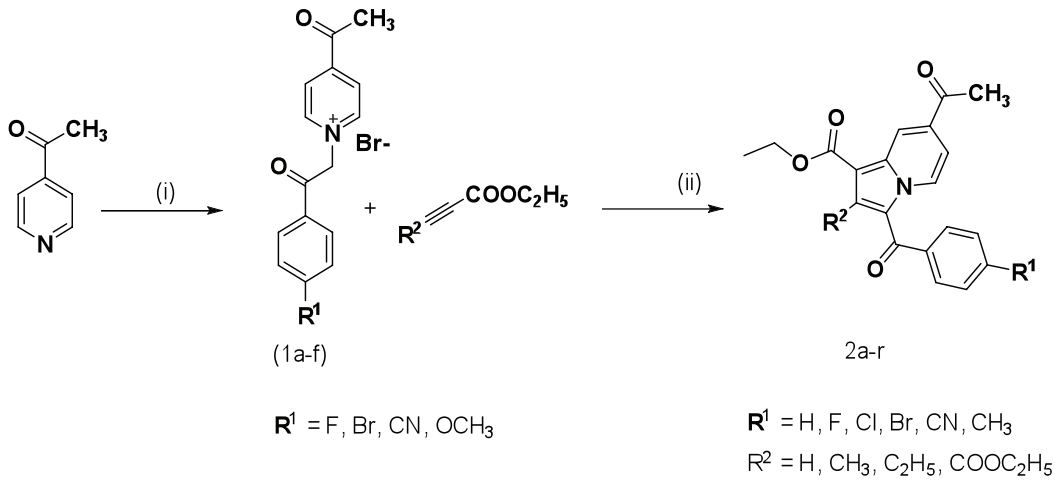

Scheme 1. Reagents and conditions: (i) 4-substituted phenacyl bromide, acetone, 5 hours stir; (ii) K_2CO_3, dimethylformamide (DMF), 30 minutes stir at room temperature.

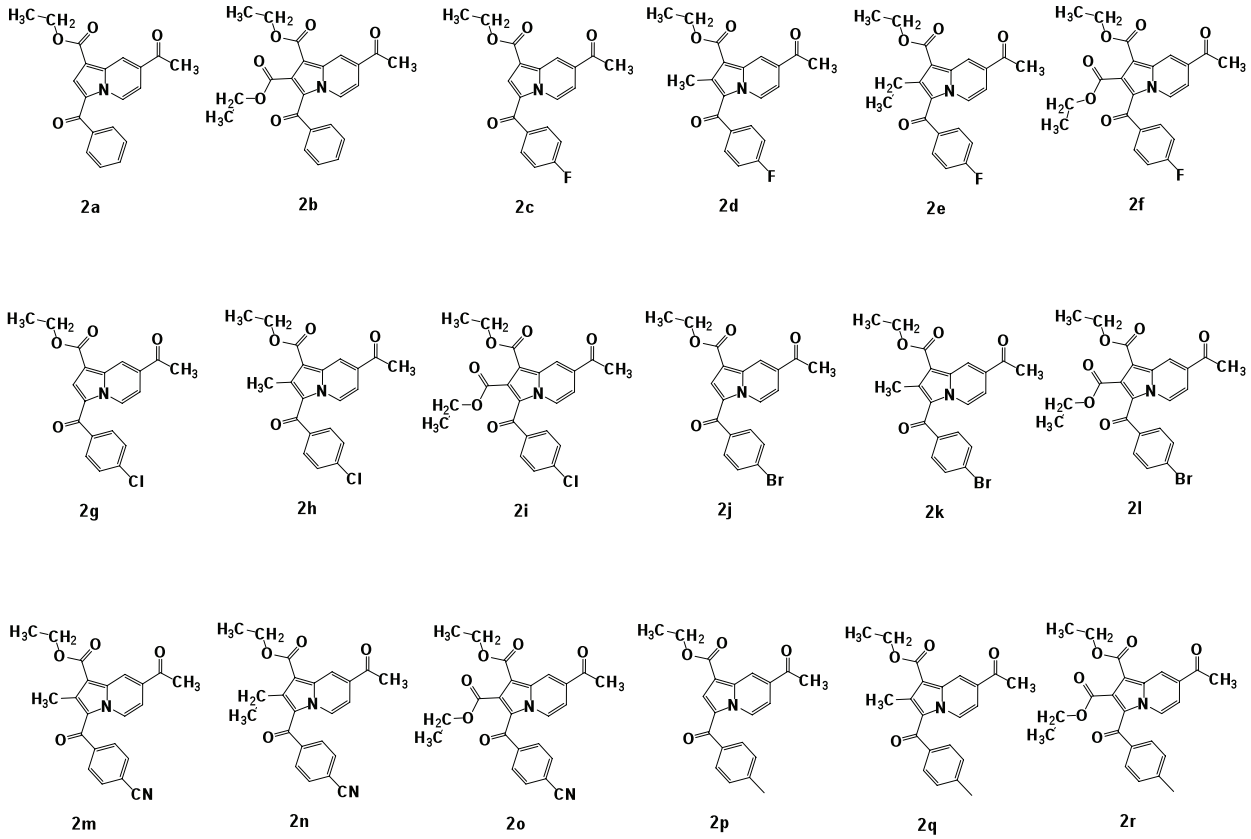

Figure 1. Molecular structure and code of indolizine analogs used for *in vitro* qualitative anti-TB activity.

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;

Table 1. Anti-tubercular activity against H37Rv strain of *M. tuberculosis*.

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

Note: [a]c Log*P* 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*., 2014a; 2014b), we evaluated synthesized (Sandeep *et al*., 2016a) 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*., 2016a). The synthetic ethyl 7-acetyl-2-substituted-3-(4-substituted benzoyl) indolizine-1-carboxylates **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*.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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