Research Article

SYNTHESIS AND COMPARATIVE ANTI-TUBERCULAR ACTIVITY OF INDOLIZINE DERIVATIVES OF ISONIAZID / PYRAZINAMIDE / ETHIONAMIDE

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ABSTRACT

Isoniazid (INH), Pyrazinamide (PZN), Ethionamide (ETN) are potent anti-tubercular agents having various side effects due to formation of toxic metabolites. The present study aims towards the prevention of these side effects and increase the efficiency of activity through pro-drug formation. Here indolizine-1-carboxylate was synthesized and combined chemically with Isoniazid (IND-INH), Pyrazinamide (IND-PZN), and Ethionamide (IND-ETN) and characterized. Anti-tubercular activity of the compounds was determined by Micro Plate Alamar Blue Assay (MABA) method against H_{37} Rv Strains of *Mycobacterium tuberculosis* at a concentration of 25 µg/ml and 50 µg /ml. Out of all synthesized derivatives only IND-INH has shown more significant anti-tubercular activity when compared to INH with dose of 50 µg/ml whereas with the dose of 25 µg/ml it has shown less significant anti-tubercular activity. The synthesized derivatives were also screened for antibacterial activity against *E.coli, P.aeruginosa, S.aureus* and *E.fecalis*. All the synthesized compounds have shown antimicrobial activity.

Keywords: Pro-drugs, Isoniazid, Pyrazinamide, Ethionamide, Indolizines.

INTRODUCTION

A number of heterocyclic compounds are known and have been shown to possess pharmacological activities. One of the important among these is that containing ring junction nitrogen. The presence of these systems is a rare occurrence in natural products, but have been a subject of many studies for preparation of potentially, biologically active analogues.

Indolizines are such parent system, which contain ring junction nitrogen and are very rare in nature. Indolizines are structurally and chemically isomeric with indoles. It is this analogy between indole and indolizine nucleus that has prompted speculation that indolizine analogs of biologically important indoles could conceivably have potent physiological activity.[1, 2]

A lot of modifications, observations and investigation have been reported in this area. Several biologically active indolizines were reported to possess biological activities like; anti-inflammatory[3], hypoglycemic[4, 5] activities. Other activities reported are $5HT_3$ receptor antagonist[6], anti-acetylcholine[7], CNS depressant activity[8], estrogen receptor binding[9], anti-oxidant property[10,11], antimicrobial[12] and analgesic activity[13]. Many amino acid derivatives with an active indolizine nucleus have been utilized in cancer therapy[14,15]. Lise-Lotte Gunderson, Ayele H. Negussie and Frode Rise synthesized various derivatives of α-hydroxybenzyl substituted indolizines which shows antimycobacterial activity[16].

Few 1-substituted indolizines have been reported to have anti-tubercular activities[17] and indolizines having hydroxyphenylmethyl or hydroxyalkyl substituents at 1-position have been found most active, but the ring also requires substituents at C-2, C-3 positions. Considering the antitubercular potential of some indolizine derivatives, it is planned to synthesize substituted indolizines and combine chemically with popular antitubercular drugs like isoniazid, Pyrazinamide, Ethionamide and study comparative antitubercular and antimicrobial activity of these derivatives.

MATERIALS AND METHODS

Synthesis of Pyridinium Halide (II)

In a conical flask 100 mmol of pyridine and 60ml of ethylacetate were taken. Added 100 mmol of chloroacetic acid and kept the conical flask on magnetic stirrer at 90°C for 2hrs. The solution turns yellowish and then kept the solution in refrigerator for about 3hrs. The solid was filtered and dried in air. The solid was recrystallized from hot methanol. The yield was 60-90% [18].

Synthesis of Indolizine-1-Carboxylate (III)

A suspension of pyridinium halide (10mmol), methyl acrylate(50mmol), triethyl amine (1.5ml) and manganese dioxide (80mmol) in toluene(80ml) were stirred at 90°C for 2hrs in round bottomed flask. After the mixture was cooled to room temperature, the brown oil was collected by distillation at 130°C. The oil was washed with water and dried on calcium chloride. The percentage yield is 57-92% [18, 19].

Synthesis of N'-(Pyridine-4-Carbonyl)Indolizine-1-Carbohydrazide (V) (IND-INH)

A mixture of indolizine-1-carboxylate (10mmole), Isoniazid (12mmole), ethanol (20ml), potassium Hydroxide (3 gm) and potassium chlorate (2 gm) was refluxed for 4 hrs at 80^{0}C. The reaction mixture was cooled to 4-5°C (for 24 hr). The solid crystals that separated were filtered, washed with cold water, dried and recrystallized from ethanol.

Synthesis of N-(Pyrazine-2-Carbonyl)Indolizine-1-Carboxamide (VII) (IND-PZN)

Indolizine-1-carboxylate (6 mmol) on treatment with pyrazinamide (8 mmol) in ethanol (20 ml) containing potassium hydroxide (3gm) refluxed for 2 hr at 80^{0}C. The reaction mixture was cooled to 4-5°C (for 24 hr). The solid crystals that separated were filtered, washed with cold water, dried and recrystallized from ethanol, gives the corresponding pyrazinamide derivative of indolizine.

Synthesis of N-(2-ethylpyridine-4-carbothioyl)indolizine-1-carboxamide (IX) (IND-ETN)

Indolizine-1-carboxylate(6mmol), ethionamide (10mmol) on reflux with 1,4-diaxone (20 ml) containing potassium hydroxide(3gm) potassium chlorate (2 gm) at 80^{0}C for 6 hrs. The reaction mixture was cooled to 4-5°C (for 24 hr). The solid crystals that separated were filtered, washed with cold water, dried and recrystallized from ethanol, gives indolizine-1-ethionamide.

Scheme- 1

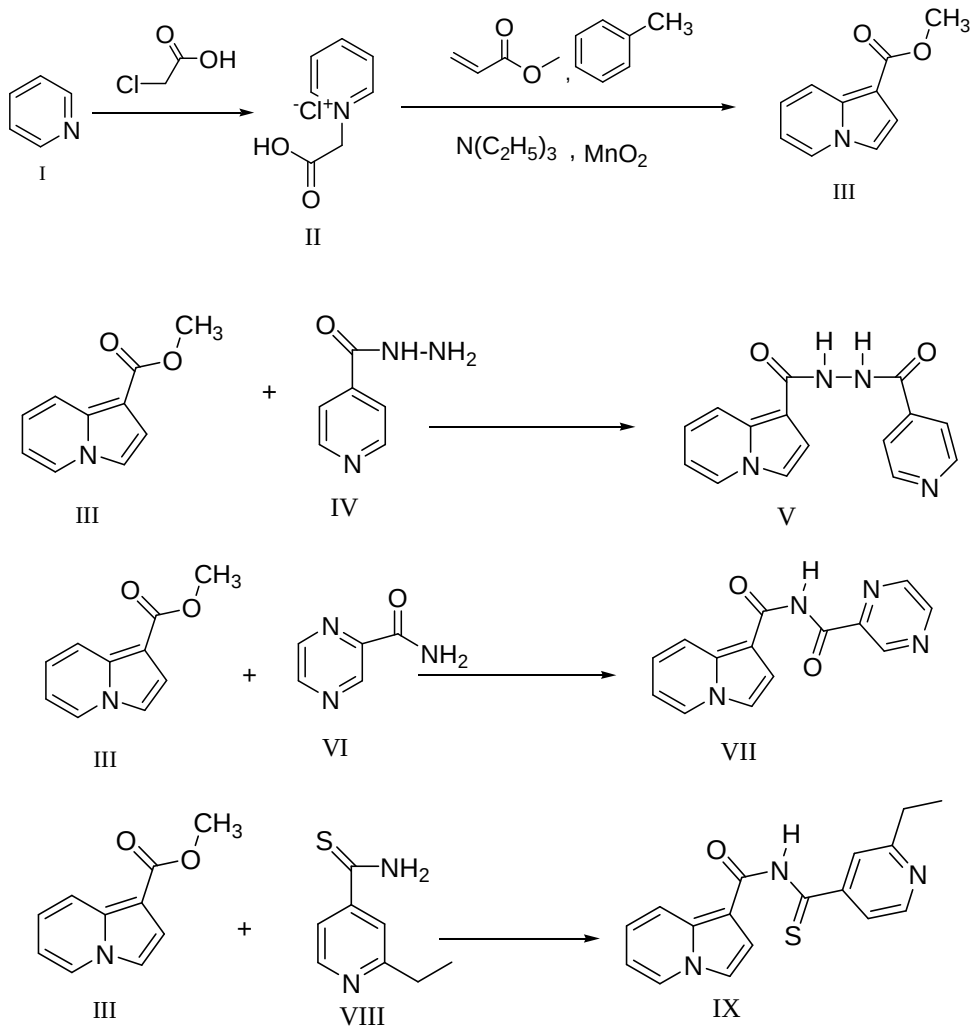


Table 1: Physical data of synthesized Indolizinyl derivatives

Compound code	Mol formula	Mol weight	Melting point (^{0}C)	Yield (%)	Rf Value
IND-INH	$C_{15}H_{12}N_4O_2$	280	**170-172**	**67.0**	**0.59**
IND-PZN	$C_{14}H_{12}N_4O_2$	268	124-125	80.0	0.63
IND-ETN	$C_{17}H_{16}N_3OS$	278	153-155	78.0	0.35

Spectral Data

1. **N'-(pyridine-4-carbonyl)indolizine-1-carbohydrazide (V) (IND-INH)**

   IR (KBr): 3379cm^{-1}(NH str), 3462.89cm^{-1}(amide NH str), 3143cm^{-1}(C-H Ar str), 1685cm^{-1}(C=NAr str), 1612, 1596cm^{-1}(secondary amide O=C-NH).

   ^{1}H NMR (DMSO-d_6): δ 8.921(s, 1H, O=C-NH), δ 8.61(s, 1H, HC=N Ar), δ 7.73-7.79(d, 2H, Ar), δ 7.37-7.45(d, 2H, Ar), δ7.25(s, 1H, Ar), δ 7.01-7.07(d, 2H, Ar).

2. **N-(pyrazine-2-carbonyl)indolizine-1-carboxamide (VII)(IND-PZN)**

   IR (KBr): 3229cm^{-1}(NH str), 3342.89cm^{-1}(amide NH str), 3063cm^{-1}(C-H Ar str), 1640cm^{-1}(C=NAr str), 1596, 1549cm^{-1}(secondary amide O=C-NH).

   ^{1}H NMR (DMSO-d_6): δ 9.91(s, 1H, O=C-NH), δ 9.21(s, 1H, HC=N Ar), δ 7.73-7.79(d, 2H, Ar), δ 7.37-7.45(d, 2H, Ar), δ7.25(s, 1H, Ar), δ 7.01-7.07(d, 2H, Ar).

3. **N-(2-ethylpyridine-4-carbothioyl)indolizine-1-carboxamide (IX)(IND-ETN)**

   IR (KBr): 3377cm^{-1}(NH str), 3466.89cm^{-1}(amide NH str), 3143cm^{-1}(C-H Ar str), 2929 cm^{-1}(C-H str), 1685cm^{-1}(C=NAr str), 1612, 1596cm^{-1}(secondary amide O=C-NH).

   ^{1}H NMR (DMSO-d_6): δ 9.921(s, 1H, O=C-NH), δ 9.31(s, 1H, HC=N Ar), δ 7.73-7.79(d, 2H, Ar), δ 7.37-7.45(d, 2H, Ar), δ7.25(s, 1H, Ar), δ 7.01-7.07(d, 2H, Ar), δ2.3(q, 2H, CH_2), δ1.59(t, 3H, CH_3).

RESULTS AND DISCUSSION

Antitubercular activity

The synthesized compounds were tested for antitubercular activity against the *Mycobacterium tuberculosis H37RV strain* using Lowenstein-Jensen Medium and Isoniazid, Pyrazinamide and Ethionamide as standard drugs. The media was prepared as per the procedure recommended by the international union against tuberculosis[20, 21]. The results obtained are presented in Fig no -1 and Fig no.2.

Figure 1: Anti-tubercular activity of synthesized derivatives against *Mycobacterium tuberculosis* H_{37} RV strain (dose of 25µg/ml).

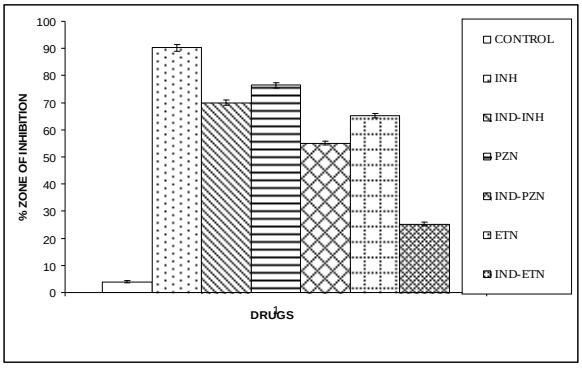
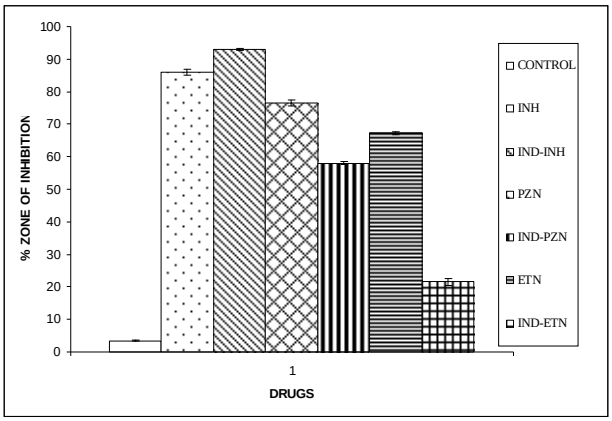


Figure 2: Anti-tubercular activity of synthesized derivatives against *Mycobacterium tuberculosis* H_{37}RV strain (dose of 50 µg/ml)



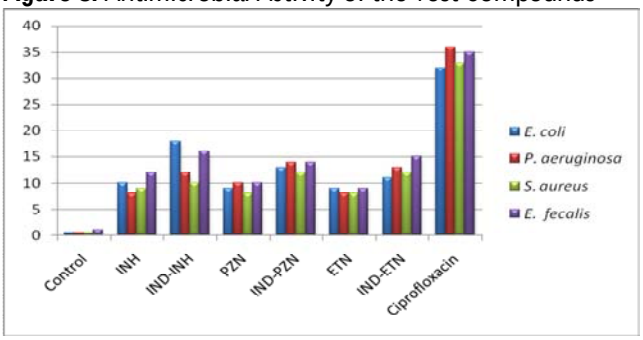
Antimicrobial activity

Antimicrobial activity of the synthesized compounds was determined, using a slightly modified cup plate method[22]. Muller Hinton agar was used for the growth of bacterial strains (*E.coli* (ATCC25922), *P.aeruginosa* (ATCC 27853), *S.aureus* (ATCC 25923) and *E.fecalis* (ATCC 29212). Each organism was suspended in normal saline solution and transmittance (T) of 75 to 77% at 530 nm was made, which is equal to 10^6 CFU/ml. All the test compounds were dissolved in DMSO at a concentration of 30 µg/ml. Each plate was inoculated with 20 µl of microbial suspension. 100 µl of the test compounds was added to each cup. The plates containing bacteria were incubated at 37^o C for 24 hrs, the positive antimicrobial activity were read based on the growth inhibition zone and compared with the solvent as control and ciprofloxacin as comparative drug, shown in Table-2. All the synthesized compounds have shown antimicrobial activity. The results are presented in the Fig no.3

Table 2: Antimicrobial Activity of the test Compounds Zone of inhibition in mm

Test Compounds	*E. coli*	*P. aeruginosa*	*S. aureus*	*E. fecalis*
Control	0.5	0.5	0.5	1
INH	10	8	9	12
IND-INH	18	12	10	16
PZN	9	10	8	10
IND-PZN	13	14	12	14
ETN	9	8	8	9
IND-ETN	11	13	12	15
Ciprofloxacin	32	36	33	35

Figure 3: Antimicrobial Activity of the Test Compounds



CONCLUSION

The synthesized derivatives inhibited the growth of *mycobacterium tuberculosis* strain ($H_{37}R_V$). Out of all synthesized derivatives only IND-INH has shown more significant anti-tubercular activity when compared to INH with dose of 50 µg/ml whereas with the dose of 25 µg/ml it has shown less significant anti-tubercular activity. IND-PZN has shown less significant anti-tubercular activity when compared to PZN with dose of 50 µg/ml and 25 µg/ml. IND-ETN has shown less significant anti-tubercular activity when compared to ETN with dose of 50 and 25 µg/ml.

The synthesized derivatives were then screened for antibacterial activity against *E.coli, P.aeruginosa, S.aureus and E.fecalis*. The results are presented in table -2. All the synthesized compounds have shown antimicrobial activity. IND-PZN has shown significant anti-microbial activity when compared to PZN.

IND-ETN has shown significant anti-microbial activity when compared to ETN. IND-INH has shown significant anti-microbial activity when compared to INH. But all the derivatives have shown very less anti-microbial activity when compared to the standard drug Ciprofloxacin.

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