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EVALUATION OF ANALGESIC AND ANTI OXIDANT ACTIVITIES OF SCHIFF BASES

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ABSTRACT

Schiff bases which contain an azomethine group attract much interest in synthetic chemistry. Schiff bases are used as substrates in the preparations of a number of industrial and biologically active compounds via closure, cyclo-addition and replacement reactions. Moreover, Schiff bases are also known to have biological activities such as antibacterial, antifungal, antitumor, antioxidant and analgesic activities. INH Schiff bases have better anti-tubercular activity than parent drug isoniazid. In the present study, three different Schiff bases of INH were prepared and the analgesic and anti-oxidant activities were investigated. Analgesic activity was evaluated by acetic-acid induced writhing test in which the Diclofenac was used as the standard drug. In vitro antioxidant activity of the INH Schiff base analogues were evaluated using DPPH free radical scavenging method. In acetic acid induced writhing test, the study showed that there was a significant inhibitory effect on writhing by all the compounds. DPPH radical scavenging activity of the three INH Schiff base analogues at different concentrations (100-500µg/ml) was compared with standard antioxidant ascorbic acid (100-500µg/ml). The compounds showed significant increase in free radical scavenging activity in a dose dependent manner.

KEYWORD: azomethine, diclofenac ,schiff base,DPPH,acetic acid.

INTRODUCTION

Historically, drugs were discovered through identifying the active ingredient from traditional remedies. Later chemical libraries of synthetic small molecules, natural products or extracts were screened in intact cells or whole organisms to identify substances that have a desirable therapeutic effect in a process known as classical pharmacology. Since sequencing of the human genome which allowed rapid cloning and synthesis of large quantities of purified proteins, it has become common practice to use high throughput screening of large compounds libraries against isolated biological targets which are hypothesized to be disease modifying in a process known as reverse pharmacology. Hits from these screens are then tested in cells and then in animals for efficacy. Even more recently, scientists have been able to understand the shape of biological molecules at the atomic level, and to use that knowledge to design drug candidates.

Modern drug discovery involves the identification of screening hits and optimization of those hits to increase the affinity, selectivity (to reduce the potential of side effects), efficacy/potency, metabolic stability (to increase the half-life), and oral bioavailability. Once a compound that fulfils all of these requirements has been identified, it will begin the process of drug development prior to clinical trials.[1]

Despite advances in technology and understanding of biological systems, drug discovery is still a lengthy, "expensive, difficult, and inefficient process" with low rate of new therapeutic discovery.[2]

SCHIFF BASE

Schiff base which contain an azomethine group attract much interest in synthetic chemistry. Schiff base with donors (N,O,S) have structural similarities with natural biological systems and important in elucidating the mechanism of transformations and reseminations reactions in biological systems due to presence of imine (- N=CH-) group.[3]

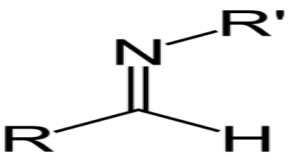


Fig 1: Schiff Base

Schiff bases are used as substrates in the preparation of a number of industrial and biologically active compounds

via closure, cyclo-addition and replacement reactions. Moreover, Schiff base are also known to have biological activities such as antibacterial[4,5], antifungal[6,7], antitumor[8,9], antioxidant[10] and analgesic activities. Schiff base complexes play a vital role in designing metal complexes related to synthetic and natural oxygen carriers[11].Metal complexes make the compounds effective as stereo-specific catalysts towards oxidation, reduction, hydrolysis biological activity and other transformations of organic and inorganic chemistry.

Similarly pyridine derivatives have been of great interest because of their role in natural and synthetic organic chemistry. Many products which contain a pyridine subunit exhibit biological activity such as antimicrobial[12] and antituberculosis activities. So the pyridine containing Schiff bases are expected to have enhanced biological activities. It is well established that the biological activity associated with the hydrazone compound attributed to the presence of the active pharmacophore (-CONH-N=C-). Hence many hydrazone compounds containing this active moiety showed good biological activities according to the literature.

ISONIAZID

Isoniazid (INH) is a drug of proven therapeutic importance and is used against a wide spectrum of bacterial ailments like tuberculosis,leprosy etc. Hydrazones derived from condensation of isoniazid with aldehydes have been found to show better antitubercular activity than INH.[13]

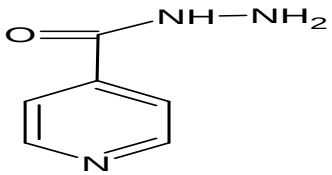


Fig 2: Isoniazid

In the past, Agarwal et al[14] have investigated the coordinating ability of INH-derivatives with metal ions. Serum concentrations of isoniazid are influenced by a number of factors,among the most important of these is the enzymatic acetylation of isoniazid by *N*-acetyltransferase (NAT). This represents a major metabolic pathway for isoniazid in human beings.

Acetylation greatly reduces the therapeutic activity of the drug. It is thought that the resultant chemical modification prevents the activation of INH that is required for proper drug action. Chemical modification of the hydrazine unit of isoniazid with a functional group that blocks acetylation, while maintaining strong action, has the potential to improve clinical out-comes and reduce the emergence in patients of acquired isoniazid resistance. The goal of our study was to investigate such chemical modification. any pilot drug derived from such chemical modification must show, at an early stage of exploration, strong activity *in vitro* and *in vivo*, low toxicity and good bioavailability.

INH SCHIFF BASE

INH schiff bases have high anti-tubercular activity than isoniazid.[15] INH hydrazones synthesized by combining INH with various aldehydes possess better anti tubercular activity and low toxicity than the parent drug INH.[16,17] Structural modification of INH by converting hydrazine moiety of the parent drug to hydrazone prevents the deactivation process of N^2-acetylation by N-aryl amino acety transferase.[10] In the present study, analgesic and anti-oxidant action of INH schiff bases were investigated.

MATERIALS AND METHODS

SYNTHESIS

Scheme of synthesis: INH Schiff bases were synthesised by the following scheme.

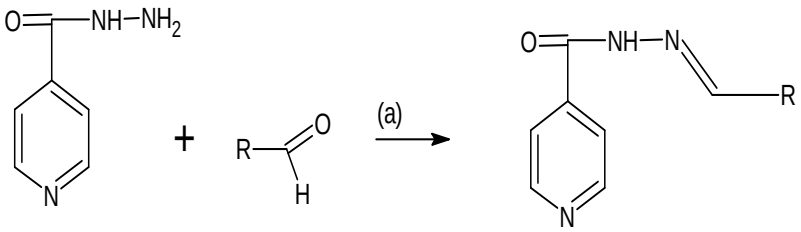


Fig : 3 scheme of synthesis

Reagents and conditions: R-substituted benzaldehyde (a)ethanol, glacial acetic acid, reflux.

General procedure for synthesis of R-(substituted)arylidene isonicotinylhydrazide

To a constantly stirred solution of INH (2.74 g, 0.02 mol) in30 mL of ethanol containing few drops of glacial acetic acid was added an appropriate aromatic aldehyde (0.02 mol). The reaction mixture was refluxed for 3 h, cooled to room temperature, and poured into crushed ice. The resulting mixture was filtered and the solid obtained was washed with cold water dried and recrystallized from rectified spirit [18]

Selection of animals

Healthy strains of Swiss albino mice were used, which is collected from Animal house, Department of Pharmacology, DPS, MGU, RIMSR, Kottayam(IAEC No.1702/PO/C/CPCSEA)

Acute toxicity study
The Institutional Animals Ethics Committee approved the use of animals for the present study and the acute toxicity was carried out as per the OECD 423 guidelines.[19]

ANALGESIC ACTIVITY *BY IN VIVO* METHOD
Acetic acid induced writhing test response in mice
Analgesic activity was evaluated by acetic-acid induced writhing test. Male albino mice (18-25g) were fasted over night with free access to water at *libitum*. Control group received only vehicle .The standard and the test group received diclofenac or test substances respectively. One hour after treatment, 1%v/v acetic acid was injected i.p (10ml/kg). Five minutes after the intraperitoneal injection of acetic acid, number of writhing were counted for the period of 20 minutes.[20]

ANTI OXIDANT ACTIVITY BY *IN VITRO* METHODS

DPPH METHOD
The free radical scavenging capacity of the extracts was determined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% methanol.

All the three Schiff bases were dissolved in DMF and diluted to prepare the stock solution 5mg/ml.

150µl of freshly prepared DPPH solution (0.004% w/v) was taken in test tubes and stock solutions of the derivatives were added followed by serial dilutions (1 µg to 500 µg) to every test tube and the final volume was made to 3 ml with methanol.[21]

After 10 min, the absorbance was read at 517 nm using a spectrophotometer.

- Ascorbic acid was used as a reference standard and dissolved in methanol to make the stock solution with the same concentration (5 mg/ml).
- Control sample was prepared containing the same volume without any derivative and reference (ascorbic acid)
- 95% methanol was used as blank.

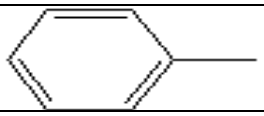
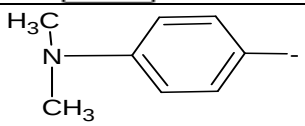
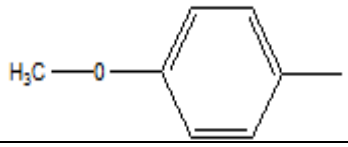
% scavenging of the DPPH free radical was measured using the following equation:-
% scavenging = (Control-test)/control x 100

Statistical analysis
The collected data on different parameters from each subject were analyzed using standard statistical techniques. The statistical tools employed in the present investigation and its purposes are summarized as below:

Descriptive Statistics
Since all the parameters are of continuous type data, the Arithmetic Mean (AM) is calculated as the representative value in the data distribution. The Standard Deviation (SD) is used for assessing the consistency of AM. If SD is small, then AM will be more accurate estimate of the parameter of interest

Analysis of Variance
Analysis of Variance (ANOVA)
ANOVA was carried out for finding statistically significant difference in the values before treatment (BT) and after treatment (AT) of each parameter among different study groups. ANOVA followed by Dunnett's multiple comparison test helps to determine the nature of BT and AT scores in different experimental groups, and also for comparing different groups with respect to each parameter.

RESULTS
Physical data of INH Schiff Bases
Table 1: Physical data of INH Schiff Bases

Compound	R	Percentage yield	Melting Point	TLC system
Schiff Base 1		80 % w/w	198°C	CHCl3:CH3OH = 4:1
Schiff Base2		85 % w/w	196°C	CHCl3:CH3OH = 4:1
Schiff Base3		87 % w/w	138°C	CHCl3:CH3OH = 4:1

Acute toxicity studies
Acute toxicity studies of INH Schiff bases were conducted as per OECD guidelines 423 using albino swiss female mice. Briefly, normal healthy mice (18 no.) were divided into groups of 6 mice in each cage. Three different INH Schiff bases, SB 1,SB2 and SB 3 were administered orally at a dose of 2000 mg/kg, p.o to (3 animals per step) each group. With freely access to food

and water, toxic symptoms and the general behavior of mice were observed continuously for 1 hr after the treatment, intermittently for 4 hr, and there after over a period of 24 hr for any signs of toxicity and mortality. The INH Schiff bases were found safe up to 2000 mg/kg. During the observation period, INH Schiff bases administration did not produce any variations in the general appearance or toxic signs in the animals.Hence the doses for further pharmacological study was fixed as 100mg/kg and 50mg/kg.

Analgesic activity

Acetic acid induced writhing

Table 2: Effect of drug treatment on acetic acid induced writhing

Treatment	Dose	No. of wriths	Inhibition (%)
Control		34±0.577	0
Indomethacin	5 mg/kg	11±0.37**	68
INH Schiff base 1	50mg/kg	26.83±0.477**	23.53
INH Schiff base 2	50mg/kg	31.16±0.307**	8.35
INH Shiff base 3	50mg/kg	27±0.577**	20.58
INH Schiff base 1	100mg/kg	20±0.365**	41.17
INH Schiff base 2	100mg/kg	24.66±0.666**	27.47
INH Schiff base 3	100mg/kg	18.66±0.333**	45.11

Values are presented as mean±S.E.M (N=6), Values are presented as mean±S.E.M (N=6), $p<0.01$=**, indicates significant increase in analgesic activity as compared with control by using ANOVA followed by Dunnett's multiple comparison test.

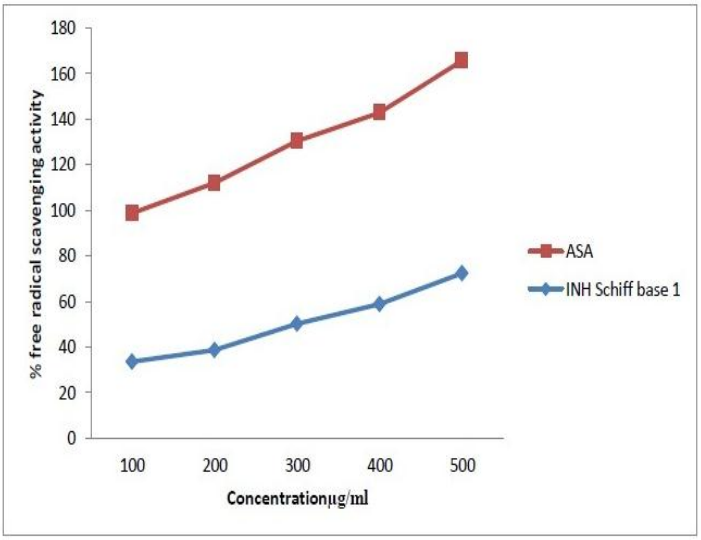
***In vitro* antioxidant action**

DPPH free radical scavenging activity

Free radical scavenging activity of INH Schiff base1

Table 3: Free radical scavenging activity of INH Schiff base1

Sl.no	Concentration(µg/ml)	% free radical scavenging activity	
		TEST	STANDARD (ASA)
1	100	33.65	65.01
2	200	38.76	73.14
3	300	50.27	80.06
4	400	58.85	83.92
5	500	72.35	93.16

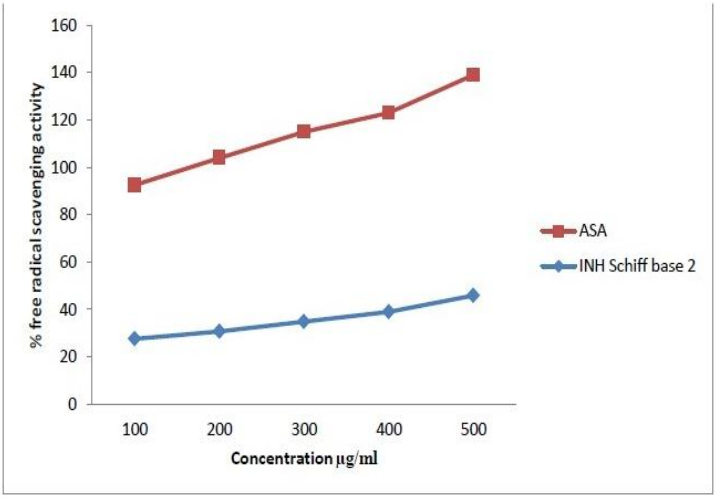


Graph 1. Free radical scavenging activity of INH Schiff base 1

Free radical scavenging activity of INH Schiff base2

Table 4: Free radical scavenging activity of INH Schiff base2

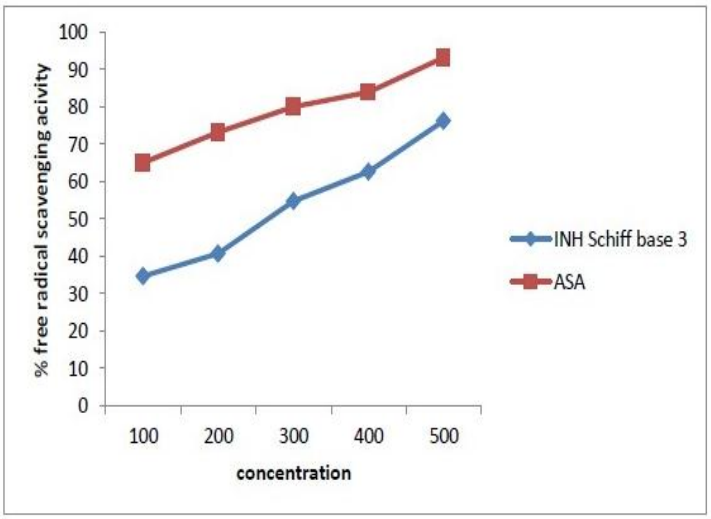
SL NO	Concentration(µg/ml)	% free radical scavenging activity	
		TEST	STANDARD (ASA)
1	100	27.45	65.01
2	200	30.65	73.14
3	300	34.76	80.06
4	400	38.97	83.92
5	500	45.70	93.16



Graph 2 Free radical scavenging activity of INH Schiff base 2
Free radical scavenging activity of INH Schiff base3

Table 5: Free radical scavenging activity of INH Schiff base3

SL NO	Concentration(µg/ml)	% free radical scavenging activity	
		Test	Standard (ASA)
1	100	34.55	65.01
2	200	40.65	73.14
3	300	54.75	80.06
4	400	62.56	83.92
5	500	76.24	93.16



Graph 3 Free radical scavenging activity of INH Schiff base 3

DISCUSSION

Schiff bases form an important class of organic compounds with a wide variety of biological properties. Development of a new chemotherapeutic Schiff bases is now attracting the attention of medicinal Chemist. Many studies have reported regarding the biological activities of Schiff bases, including their anticancer, antibacterial, antifungal, and herbicidal activities. Schiff bases, derived from various heterocycles were reported to possess cytotoxic, anticonvulsant, antiproliferative, anticancer, and antifungal activities.[4,5,6,7,8,9,10] In the present study the analgesic activity of three INH schiff base analogues are evaluated by in vivo method along with in vitro antioxidant activity.

In vitro antioxidant activity of the three INH Schiff base analogues were evaluated using DPPH free radical scavenging method. The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1 Diphenyl-2-Picryl Hydrazine. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants95. Hence the colour change from deep purple to yellow colour was measured at 517nm96. The DPPH radical scavenging activity of the three INH Schiff base analogues at different concentrations (100-500µg/ml) was compared with standard antioxidant, Ascorbic acid at varying concentrations (100- 500µg/ml). The compounds showed significant increase in free radical scavenging activity in a dose dependent manner.INH Schiff base 1 and INH Schiff base3 showed better antioxidant property with IC50 value of 297µg/ml and 285µg/ml respectively and INH Schiff 2 showed lesser antioxidant activity with IC50 value of greater than 500µg/ml (table1,2 & 3). Hence compound 1 and 3 possess considerable antioxidant properties.

CONCLUSION

The present study was carried out to investigate the analgesic and anti oxidant activity of INH Schiff bases (INH Schiff base 1, INH Schiff base 2, INH Schiff base 3).

In conclusion, our investigations and data obtained from this study demonstrated that INH Schiff base1 and INH Schiff base 3 possessed very good analgesic and anti oxidant activity while INH Schiff base 2 has comparatively less activity comparing with other two.

In acetic acid induced writhing test, the study showed that there was a significant inhibitory effect on writhing by all the compounds.The screening of these INH Schiff bases for analgesic activity were carried in mice by acetic acid induced writhing reflex In acetic acid induced writhing test, all the compounds significantly inhibited the number of wriths and possessed peripheral analgesic activity, which was comparable to the effect produced by the standard drug diclofenac sodium. Hence the results obtained from this studies support that INH Schiff base 1 and 3 have good antioxidant and analgesic effect but further studies are required to prove the exact mechanism of action of this compounds.

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