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Synthesis, Antitubercular Activity and Pharmacokinetic Studies of Some Schiff Bases Derived from 1- Alkylisatin and Isonicotinic Acid Hydrazide (INH)

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N-(1-alkyl-2,3-dihydro-2-oxo-1*H*-3-indolyliden)-4-pyridinecarboxylic acid hydrazide derivatives, **3(a-g)**, were synthesized in a trial to overcome the resistance developed with the therapeutic uses of isoniazid (INH). The lipophilicity of the synthesized derivatives supersedes that of the INH as expressed by Clog p values. The synthesized compounds and INH were tested against bovin, human sensitive and human resist strains of Mycobacterium tuberculosis. Compounds **3a**, **3d**, **3f** and **3g** with 1-unsubstituted, 1-propyl, 1-propynyl and 1-benzyl groups respectively exhibited equipotent growth inhibitory activity (MIC 10 μmol) against the tested strains as compared with INH however the later has no activity against human resist strain. Pharmacokinetic study revealed that the rate and extent of absorption of the tested derivatives (**3d** and **3f**) significantly higher than that of INH ($p<0.05$). The relative bioavailabilities (F_R%) were 183.15 and 443.25 for **3f** and **3d** respectively as compared to INH. These results preliminary indicate the possible use of the prepared derivatives for treatment of tuberculosis infections in order to overcome the resistance developed with INH.

Key words: 1-Alkylisatin, Schiff bases, Lipophilicity, Antimycobacterial, *In-vivo* bioavailability, Relative bioavailability

INTRODUCTION

Drug resistance and multidrug-resistant *tuberculosis* is perceived as a growing hazard to human health worldwide. TB ranks among the most important burdens on human health, not only due to the large number of cases (~9 million/ year worldwide, with incidence rates typically measured per 100,000 population), but also because about one quarter of sufferers die, most of them young adults. Globally, the number of TB cases is currently rising at 2%/year. The fear is that the number of cases resistant to antitubercular drugs may be increasing much faster (Dye *et al.,* 2002, 2000; Pablos-Mendez *et al.,* 2002; Petrini *et al.,* 1999). The perceived threat of drug-resistant TB is enormous. The biggest menace is multidrug-resistant TB caused by strains resistant to at least INH and rifampicin, the two principal first line drugs used in combination chemotherapy. The major concerns over drug resistance were a fear of the spread of drug-resistant organisms and the ineffectiveness of chemotherapy in patients infected with them. If these spread increasingly in a community, TB may become progressively uncontrollable using currently available chemotherapy. One of the strategies suggested for overcoming this problem is the fully exploiting the potential of standard short course chemotherapy based on cheap and safe first line drugs. Furthermore, the development of potent new antitubercular drugs without cross-resistance with known antimycobacterial agents is urgently needed (Tomioka 2002).

On the other hand, isatin (indolin-2,3-dione) derivatives are reported to show variety of biological activities like antibacterial (Daisley *et al.*,1984), antifungal (Piscopo *et al.*, 1987), antiviral (Masgalith *et al.*, 1976) anti-HIV (Selvam *et al.*, 2001) and antitubercular activities (Brown *et al.*, 1956; Buu-Hoi *et al.*, 1953). Accordingly isatin is a versatile lead molecule for designing of potential antimicrobial agent. In view of the antimicribial property of this pharmacophore

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it was envisaged that its combined effect with an active moiety may result in increased antimicribial activity. Furthermore, it was suggested that the mechanism of resistance to INH is related to failure of the drug to penetrate or to be taken up by the microorganisms (Mondell *et al.*, 1991). Fortunately, pharmacokinetic properties and cellular permeability of a drug can be modulated by its derivatization to more lipophilic forms (Conradi *et al.*, 1996).

Thus the present work involves incorporation of INH with isatin or its 1-alkyl derivatives and *in vitro* evaluation of antitubercular activity of these synthesized compounds in comparison with INH. Furthermore pharmacokinetic investigations for representative compounds were also undertaken.

MATERIALS AND METHODS

Isonicotinic acid hydrazide (INH) was a gift from Chemical Industries Development Co, CID, Cairo, Egypt. All other chemicals were of commercial grade except the HPLC solvents and the buffer reagents were of analytical grade.

Melting points were determined on an electrothermal melting point apparatus [Fa. Sturat Scientific, England], and were uncorrected

Precoated silica gel plates (kiesel gel 0.25 mm, 60G F254, Merk) were used for thin layer chromatography. Developing solvent system of chloroform/ methanol (90: 10) was used and the spots were detected by ultraviolet light.

Products were percolated on a column chromatography packed with silica gel 60 (particle size 0.06-0.2 mm) and eluted with the same TLC developing system.

^{1}H-NMR Spectra were recorded with an IBM AF200 MHz FT-NMR, USA. Chemical shifts are expressed in δ (ppm) relative to TMS as an internal standard.

Elemental analyses were performed at the Department of Chemistry, Faculty of Science, Assiut University, Assiut, Egypt.

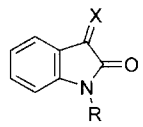
HPLC system is consisting of a pump (Gilson pump 162829, France), a Synchroback reversed-phase HPLC column (250×4.6 mm), a variable-wavelength detector (Gilson detector 109D6CO66, USA), a Hewlett Packard recording integrator (France) and a 20 µL injection loop. The mobile phase was composed of methanol and phosphate buffer (70:30 v/v, pH 6). The column effluent was monitored at 237 nm, the flow rate was 1 mL/min and integration was done by using sit at 10 mm/min. Quantitation of the eluted compounds was done from peak height measurements in relation to those of standards chromatographed under the same conditions.

Antitubercular activity was performed at the Department of Microbiology & Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt. The tested *Mycobacterium tuberculosis* strains (bovin, human sensitive and human resist) were gifted by the Department of Microbiology & Immunology, Faculty of Medicine, Assiut University.

Synthesis of 1-alkyl isatin (2a-e)

To a stirred solution of isatin (1 mmol) in DMF (10 mL), potassium carbonate (1.5 mmol) was added followed by appropriate alkyl bromide (1.1 mmol). The reaction mixture then heated while stirring to 80°C for 8 h. The reaction mixture flittered and the filtrate evaporated under reduced pressure to dryness. The residue was percolated on silica gel column and the product then crystallized from the suitable solvent. Yields and melting points are given in Table I. ^{1}H-NMR data are given in Table II.

Table I. Some physicochemical data of the synthesized derivatives **2a-f** and **3a-g**



Compd.	R	X	Yield (%)	Formula	M.P./°C (Cryst. Solvent)	Clog P[a]
2a	CH_3	O	80	$C_9H_7NO_2$	130-133 (ethanol)	
2b	C_3H_7	O	75	$C_{11}H_{11}NO_2$	198 (pet. Ether)	
2c	C_3H_5	O	60	$C_{11}H_9NO_2$	88-9 (cyclohexan)	
2d	C_3H_3	O	65	$C_{11}H_7NO_2$	122 (ethanol)	
2e	$C_6H_5CH_2$	O	72	$C_{15}H_{11}NO_2$	131-2 (ethanol)	
2f	CH_2OH	O	70	$C_9H_7NO_3$	150-152 (ethyl acetate)	
3a	H	N-R[(b)]	95	$C_{14}H_{10}N_4O_2$	293-5 [c] (aq. methanol)	2.106
3b	CH_3	N-R	90	$C_{15}H_{12}N_4O_2$	220 [d] (methanol)	2.982
3c	C_3H_7	N-R	85	$C_{17}H_{16}N_4O_2$ [e]	198-200 (ethanol)	4.040
3d	C_3H_5	N-R	75	$C_{17}H_{14}N_4O_2$ [e]	161-164 (ethanol)	3.556
3e	C_3H_3	N-R	70	$C_{17}H_{12}N_4O_2$ [e]	204-207 (ethanol/EoAc)	2.682
3f	$C_6H_5CH_2$	N-R	80	$C_{21}H_{16}N_4O_2$ [e]	285 (ethanol/pet.ether)	4.55
3g	CH_2OH	N-R	85	$C_{15}H_{12}N_4O_3$ [e]	247-50 (ethanol)	0.846

[a] Clog P value for INH is -0.708, Reported -0.700 (Leo, 1993).
[c] Reported m.p. 292-3°C (El-Sebai *et al.*, 1973).
[d] Reported m.p. 219°C (Somogyi, 2001).
[e] Elemental analyses for C,H,N, are within±0.4%.

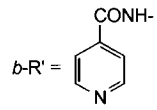


Table II. ^{1}HNMR spectral data of the synthesized derivatives **2a-f**[a] and **3a-g**[b]

Compd.	Chemical Shifts, δ ppm, (J=Hz)
2a	3.26 (3H, s, NCH_3), 6.90 (1H, t, J = 3.3, C_7H), 7.13 (1H, t, J = 5, C_5H), 7.59-7.62 (2H, m, C_4H & C_6H)
2b	0.96 (3H, t, J = 7.4, $NCH_2CH_2\underline{CH_3}$), 1.72-1.79 (2H, m, $CH_2\underline{CH_2}CH_3$), 3.69 (2H,t, J = 7.5, $\underline{CH_2}CH_2CH_3$), 6.90 (1H, d, J = 5, C_7H), 7.13 (1H, t, J = 4.5, C_5H), 7.56-7.62 (2H, m, C_4H & C_6H)
2c	4.36-4.38 (2H, m, $N\underline{CH_2}CHCH_2$), 5.28-5.36 (2H, m, $CH_2CH\underline{CH_2}$), 5.81-5.90 (1H, m, $CH_2\underline{CH}CH_2$), 6.90 (1H, d, J = 4.8, C_7H), 7.11 (1H, t, J = 4.8, C_5H), 7.55-7.63 (2H, m, C_4H & C_6H)
2d	2.3 (1H, t, J = 1.4, $NCH_2C\underline{CH}$), 4.54 (2H, d, J = 1.5, $\underline{CH_2}CCH$), 7.13 (1H, d, J = 5.8, C_7H), 7.18 (1H, t, J = 4.5, C_5H), 7.63-7.67 (2H, m, C_4H & C_6H)
2e	4.99 (2H, s, $N\underline{CH_2}C_6H_5$), 6.78 (1H, d, J = 4.8, C_7H), 7.1 (1H, t, J = 4.8, C_5H), 7.26-7.41 (5H, m, $CH_2\underline{C_6H_5}$) 7.48 (1H, t, J = 4.8, C_6H), 7.62 (1H, d, J = 5.5, C_4H)
2f	5.07 (2H, d, J = 7, $N\underline{CH_2}OH$), 6.32 (1H, t, J = 7, $CH_2\underline{OH}$), 6.91 (1H, d, J = 5, C_7H), 7.11 (1H, t, J = 4.8, C_5H), 7.52-7.60 (2H, m, C_4H & C_6H)
3a	6.89 (1H, d, J = 5, C_7H), 7.08-7.11 (1H, m,C_5H), 7.37-7.42 (2H, m, C_4H & C_6H), 7.59 (1H, s, NH), 7.77 (2H,d, J = 3.5, 3,5 pyr. H), 8.78 (2H,d, J = 3.8, 2,6 pyr. H), 11.89 (1H,s,CO$\underline{NH}$)
3b	3.3 (3H, s, $N\underline{CH_3}$), 7.16-7.19 (2H, m, C_7H& C_5H), 7.5 (1H, t, J = 4.8, C_6H), 7.60-7.68 (1H, m, C_4H), 7.78 (2H,d, J = 4, 3,5 pyr. H), 8.86 (2H,d, J = 4, 2,6 pyr. H)
3c	5.18 (2H, d, J = 7, $N\underline{CH_2}OH$), 6.52 (1H, t, J = 7, $CH_2\underline{OH}$), 7.19 (1H, d, J = 4.5, C_7H), 7.28 (1H, d, J = 5, C_5H), 7.48 (1H, t, J = 4.8,C_6H), 7.62-7.68 (1H, m, C_4H), 7.79 (2H,d, J = 4, 3,5 pyr. H), 8.85 (2H,d, J = 4, 2,6 pyr. H)
3d	0.90 (3H, t, J = 7.4, $NCH_2CH_2\underline{CH_3}$), 1.62-1.70 (2H, m, $CH_2\underline{CH_2}CH_3$), 3.73 (2H,t, J = 7.5, $\underline{CH_2}CH_2CH_3$), 7.16 (1H, t, J = 5, C_7H), 7.24 (1H, d, J = 5, C_5H), 7.48 (1H, t, J = 4.8,C_6H), 7.62-7.68 (1H, m, C_4H) 7.78 (2H,d, J = 5, 3,5 pyr. H), 8.85 (2H,d, J = 4, 2,6 pyr. H)
3e	4.41 (2H, d, J = 5.5, $N\underline{CH_2}CHCH_2$), 5.18-5.28 (2H, m, $CH_2CH\underline{CH_2}$), 5.8.2-5.94 (1H, m, $CH_2\underline{CH}CH_2$), 7.13 (1H, d, J = 5, C_7H), 7.18 (1H, t, J = 5, C_5H), 7.46 (1H, t, J = 4.8, C_6H), 7.62-7.70 (1H, m, C_4H) 7.79 (2H,d, J = 3.8, 3,5 pyr. H), 8.91 (2H,d, J = 4, 2,6 pyr. H)
3f	2.48 (1H, t, J = 1.3, $NCH_2C\underline{CH}$), 4.66 (2H, d, J = 1.3, $N\underline{CH_2}CCH$), 7.22 (1H, t, J = 5, C_7H), 7.27 (1H, d, J = 5,C_5H), 7.53 (1H, t, J = 4.8, C_6H), 7.66 (1H, m, C_4H), 7.79 (2H,d, J = 3.8, 3,5 pyr. H), 8.86 (2H,d, J = 3.8, 2,6 pyr. H)
3g	5.01 (2H, s, $N\underline{CH_2}C_6H_5$), 7.08 (1H, d, J = 4.8, C_7H), 7.16 (1H, t, J = 4.5, C_5H), 7.25-7.41 (6H,m, $CH_2\underline{C_6H_5}$&C_6H) , 7.64-7.68 (1H, m, C_4H), 7.86 (2H,d, J = 4, 3,5 pyr. H), 8.86 (2H,d, J = 4, 2,6 pyr. H)

[a] $CDCl_3$.
[b] DMSO-d_6.

Synthesis of 1-hydroxymethyl isatin (2f)

A suspension of isatin (1 mmol) and 40% formalin solution (3 mL) in water (10 mL) was refluxed for 2 h. The resulting solution was filtered while hot and the filtrate cooled and left for overnight. The formed crystalline product was filtered, dried and recrystallized from ethyl acetate. Yield and melting point are given in Table I. ^{1}H-NMR data are given in Table II.

Synthesis of *N'*-(1-alkyl-2,3-dihydro-2-oxo-1*H*-3-indolyliden)-4-pyridinecarboxylic acid hydrazide (3a-g)

Isatin or its 1-alkyl derivatives, **2a-f**, (0.5 mmol) and isonicotinic acid hydrazide (0.5 mmol) were refluxed in ethanol (25 mL) in presence glacial acetic acid (1 mL) for 4 h. The resulting solid was filtered, washed with water, dried and recrystallized from appropriate solvent. Yields, melting points and physical data are given in Table I. ^{1}H-NMR data are given in Table II.

Calculation of log P values

The log P values of the synthesized derivatives as well as the parent compound, INH, were computed with a routine method called calculated log P (Clog P) contained in a PC-software package (MacLogP 2.0, BioByte Corp., CA, USA). A representation of the molecular structure where hydrogens are omitted, or suppressed (SMILES notation), is entered into the program, which computes the log P based on the fragment method developed by Leo (Leo 1993), Results are given in Table I.

In vitro evaluation of the antitubercular activity of the synthesized compounds

The anti-TB activity of the tested compounds were carried out using Rist and Grosset proportion method (Canetti *et al.*, 1983).

The synthesized compounds, **3a-g**, and the INH, were solubelized in DMSO at a concentration of 1 mmol. Appropriate amounts of each compound was diluted with Lowenstein-jensen media to give a concentrations of 25, 10, 5 and 2.5 μmols. The media containing different compounds with various concentrations were inspissated at 70°C for one hour in hot air oven for three successive days. The sterilized media were then inoculated by 10^{-3} and 10^{-5} dilutions of the reference strains [Bovin T.B., Human resist and Human sensitive T.B. reference strains]. The minimum inhibitory concentration (MIC) of the tested compounds were evaluated after incubation at 37°C for six weeks. Each batch of tests included a control experiment using the standard strain of Bovin T.B., Human resist and Human sensitive T.B. in a media free from drugs. Results are given in Table III.

Bioavailability studies

The bioavailability of INH, **3d** and **3f** was performed using a three-way-crossover study on six rabbits weighing 1.8-2 kg. The tested compounds were administered orally at a dose of 25mg/kg dissolved (**INH**) or suspended in 5 mL of distilled water by sonicattor (**3d** and **3f**). Blood samples (2 mL) were withdrawn from the eye vein into heparine tubes before drug administration (zero time) and at 0.25, 0.5, 1, 2, 3, 5, 8 and 24 h following drug administration. Plasma was immediately separated by centrifugation and stored at -20°C until analysis.

HPLC analysis

INH, **3d** and **3f** were determined in plasma by the following analytical procedure: To 500 μL plasma containing 2 μg of the internal standard (**3f** was used as internal standard for INH and **3d** while the later was used as internal standard for **3f**), 500 μL methanol was added and vortex mixed. Extraction was done with 5 mL chloroform by vortex and centrifugation for 10 min. at 5000 rpm. The organic layer was evaporated at 45°C in a water bath. The residue was reconstituted in 500 μL mobile phase, filtered and 20 μL was injected into HPLC apparatus.

The method was linear between the tested range of concentrations ($r > 0.99$) for INH, **3d** and **3f**. The within-day coefficients of variation (CV %) of the assay ranged from 1.55 to 7.36%, from 2.66 to 9.66% and from 3.77 to 11.36% for INH, **3d** and **3f**, respectively. The overall relative standard deviations (RSD%) were $2.33\pm1.2\times10^{-3}$, $6.77\pm4.5\times10^{-3}$ and $6.55\pm2.3\times10^{-3}$ for INH, **3d** and **3f**, respectively. The between-day coefficients of variation (CV %) ranged from 3.2 to 8.66, from 1.99 to 12.63% and from 2.54 to 12.69 for INH, **3d** and **3f**, respectively. The overall relative standard deviations (RSD %) were $4.56\pm3.54\times10^{-3}$, $3.65\pm4.50\times10^{-3}$ and $4.65\pm3.69\times10^{-3}$ for INH, **3d** and **3f**, respectively. The overall mean absolute recoveries for INH, **3d** and **3d** were 96.54±4.66%, 99.42±1.23% and 98.45±2.89%, respectively, indicating the efficiency of the assay method.

Pharmacokinetic parameters

A computer program (R-Strip) was used to calculate the pharmacokinetic parameters. The peak plasma concentration (C_{max}) and time of the peak (t_{max}) were determined from the plasma concentration-time profiles. The elimination half life ($t_{0.5\ ele.}$), overall elimination rate constant (K), absorption half-life ($t_{0.5\ abs}$), absorption rate constant (K_a), $AUC_{0\text{-}24h}$ (area under the plasma concentration time curve from zero time to 24 h), $AUC_{0\text{-}8}$ (area under the plasma concentration time curve from zero time to infinity), $AUMC_{0\text{-}24h}$ (area under first moment time curve from zero time to 24 h), $AUMC_{0\text{-}\infty}$ (area under first momenttime curve from zero time to infinity), Vd (volume of distribution), Cl_T (total body clearance) and F_R (relative bioavailability of the prepared compounds to INH) The relative bioavailability (F_R) was determined by dividing the $AUC_{0\text{-}\infty}$ of any preparation by that of the reference (INH).

The area under the plasma concentration time curve (AUC) and the area under first moment-time curve (AUMC) were calculated using the linear trapezoidal rule. The plasma concentration was determined from the following equation (Niazi, 1992; Shargel *et al.*, 1993):

$$C_p = \frac{Fk_aD_o}{(k_a-k)Vd}(e^{-kt}-e^{-kat}) \quad (1)$$

where C_p = plasma concentration at time t, K = overall elimination rate constant, K_a = absorption rate constant, D_o = dose administered rectally or orally and F = fraction absorbed systemically.

In post absorptive phase, k_a~zero, and C_p is given by equation 2:

$$C_p = \frac{Fk_aD_o}{(k_a-k)Vd}e^{-kt} \quad (2)$$

where k was determined from the terminal half-life and k_a by using the method of residual from the semilog plot of C_p versus t (Niazi, S. 1992; Shargel L., *et al.*, 1993).

Statistical analysis

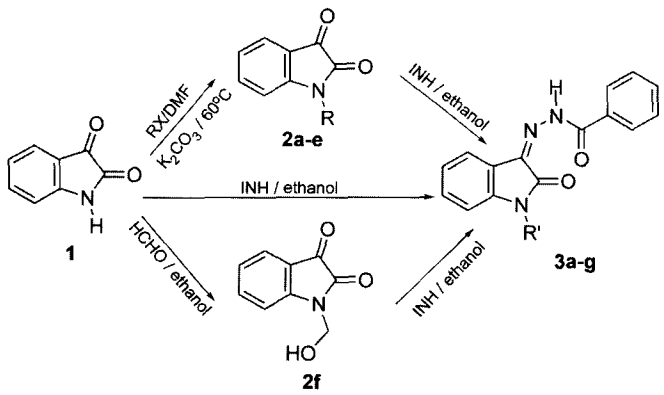
Statistical analysis of the pharmacokinetic parameters was carried out using un-paired student *t*-test ($P<0.05$).

RESULTS AND DISCUSSION

Chemistry

The target compounds, **3b-g**, were synthesized in a two step reaction. The first step involved preparation of *N*-alkylisatins, **2a-e**, by reaction of indolin-2,3-dione (**1**) with alkyl halides in the presence of anhydrous potassium carbonate according to the reported procedure of Radul *et al.* (Radul *et al.*, 1983). However, 1-hydroxymethylisatin, **2f**, was obtained by the reaction of isatin with formaline. The physicochemical properties and ^{1}H-NMR spectral data of these intermediates were given in Table I and II respectively and were matched with the reported ones (Knotz *et al.*, 1970; Mahfouz *et al.*, 1999; Majumdar *et al.*, 1996). The second step, however, involved the reaction of isatin or its *N*-alkyl derivatives (**2a-f**) with INH in boiling ethanol and in presence of acetic acid to provide the targeted derivatives, **3a-g**, Scheme 1 and Table I.

Structures of these synthesized compounds were verified on the bases of spectral and elemental methods of analyses. ^{1}H-NMR spectra were consistent with the assigned structures (Table II). The *N*-alkylation does not alter significantly the chemical shifts of isatin moiety protons (Silva *et al.*, 2001). A downfield shift of 0.10-0.18 ppm,



Scheme 1. R=a, CH_3 b,n-C_3H_7; c, allyl; d, propygyl; e, benzyl. R'=a, H; b, CH_3; c, CH_2OH; d, *n*-C_3H_7; e, allyl; f, propygyl; g, benzyl.

however, was observed with the chemical shift of H-5 of *N*-alkyl derivatives, **2a-f**, but the coupling pattern perturbed in all the protons of *N*-alkyl derivatives compared with those of isatin. In case of Schiff base derivatives (**3b-g**) a downfield shift of 0.16-0.22 ppm was observed with the chemical shifts of H-4, H-5 and H-7 compared with the reported chemical shifts of these sets of protons for the unsubstituted isatin.

Lipophilicity

Lipophilicity of the synthesized derivatives, **3a-g**, and the parent compound, INH, is expressed in the term of their log P values. These values were computed with a routine method called calculated log P (Clog P) contained in a PC-software package described in experimental section. Computation of the log P is based on the fragment method developed by Leo (Leo, 1993).

As shown in Table I, a remarkable improvement in the lipophilicity of the synthesized derivatives, **3a-g**, compared with the parent drug, INH. This may be rendering them more capable of penetrating various biomembrane (Møss *et al.*, 1990), consequently improving their permeation properties toward mycobacterial cell membrane (Seydel, *et al.*, 1995). In other wards, the improvement of the lipophilic character of the synthesized derivatives probably enhances their bioavailability to the requested site of action. This in turn participates in overcoming the resistance developed from the failure of the drug to penetrate the microorganisms.

Antitubercular activity

The synthesized compounds, **3a-g**, were tested for their antimycobacterial activity *in vitro* using **3** different strains of *Mycobacterium tuberculosis* [Bovin, Human sensitive and Human resist T.B.] according to the protocol described in the experimental section. Results of the *in vitro* antitubercular activity of the tested compounds are given in Table III. Control experiments were done using a growth media free from drugs.

Table III. *In vitro* antitubercular activity of the synthesized compounds in comparison with INH

T.B. Strain	MIC (μg/mL) of Tested compounds[a]				
	3a	**3d**	**3f**	**3g**	**INH**
Bovin	2.7	3.1	3.5	3	1.4
Human Sensitive	2.7	3.1	3.5	3	1.4
Human Resist	2.7	3.1	3.5	3	-

[a] Approximated to one decimal place

The obtained results revealed that compounds **3a**, **3d**, **3f** and **3g** exhibited equipotent growth inhibitory activity against the tested *Mycobacterium tuberculosis* strains to that obtained with INH, however, the later has no activity against human resist strain. This is may be attributed in part for the improvement of the permeation properties of the synthesized compounds toward mycobacterial cell membrane relative to INH. On the other hand, in spite of the enhanced lipophilic properties of compounds **3b**, **3c** and **3e**, they were completely inactive against the tested strains at the investigated dose levels. This result indicated that the lipophilicity of the tested compounds is not the sole parameter affecting their antitubercular activity.

Pharmacokinetic study

In vivo bioavailability of two representatives of the active derivatives, **3d** and **3f**, has been studied in comparison with INH according the protocol mentioned in the experimental part. Fig. 1 Shows typical chromatograms of tested compounds (INH, **3d** and **3f**) at concentration of 4 μg/mL of each drug. The chromatograms characterized by sharp and symmetrical peaks which indicate excellent resolution. The retention time for INH, **3d** and **3f**, were

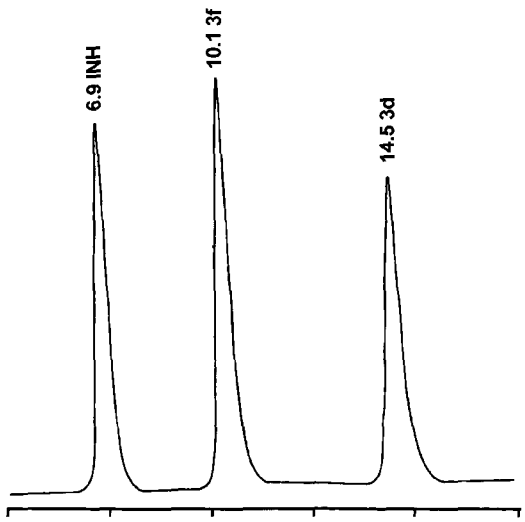


Fig. 1. Typical HPLC chromatogram of INH, **3d** and **3f** at a concentration of 4 μg/mL

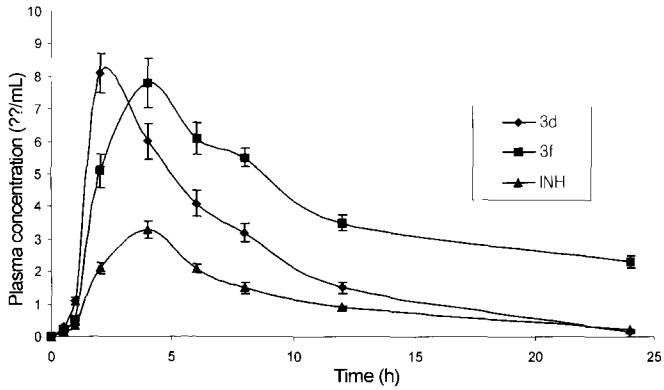


Fig. 2. Plasma concentration-time profile of INH, **3d** and **3f** following oral administration (25 mg/kg) to rabbits.

6.9, 14.5 and 10.1 minutes respectively (Fig. 1).

Fig. 2 shows the plasma concentration time profiles of INH, **3d** and **3f** following oral administration (25 mg/kg) of the tested drugs to six rabbits. The obtained results showed rapid absorption of **3d** and **3f** as compared to INH that suggest the possibility of enhancing the activity of **3d** and **3f**. The improved absorption of **3d** and **3f** as compared to INH could be attributed to the increased lipophilicity of the prepared drugs (Table I). Table IV summarizes the calculated pharmacokinetic parameters for the tested derivatives (**3d** and **3f**) in comparison with INH.

The mean values of the maximum plasma concentrations (C_{max}) were 8.1±1.20 and 7.80±1.1 μg/mL for 3d and 3f, respectively compared to 3.32±0.31 μg/mL for INH (Table IV). The corresponding values of t_{max} (time to reach C_{max}) were 3.3±0.15 and 3.6±0.31 h for **3d** and **3f**, respectively compared to 5.4±0.64 h for INH (Table IV). The shorter t_{max} and higher C_{max} values of the prepared derivatives as compared to INH could be attributed to the increase of their lipophilicity (Table I). This could be also the reasons for the observed antitubercular activity of the active synthesized derivatives against human resist T.B. strain (Table III).

The mean values of the absorption rate constant (K_a) were 0.40±0.03 and 0.45±0.05 h^{-1} for **3d** and **3f**, respectively compared to 0.18±0.02 h^{-1} for INH (Table IV). Again the higher K_a value of the prepared derivatives as compared to INH could be attributed to the increased lipophilicity of these derivatives (Table I).

There were significant differences ($p<0.05$) in all the pharmacokinetic parameters of the tested derivatives as compared to INH (Table IV). The relative bioavailabilities (F_R %) were 183.15 and 443.25 for the **3d** and **3f**, respectively as compared to INH (Table IV). These results clearly indicate the possible use of the prepared deriva-

Table IV. Pharmacokinetic parameters of **3d**, **3f** and INH following oral administration to rabbits[a]

Pharmacokinetic Parameters [b]	Compound			Statistics[c] ($p<0.05$)
	3f (A)	**3d** (B)	**INH** (C)	
C_{max} (μg/mL)	7.80 ± 1.10	8.10 ± 1.20	3.32± 0.31	A=B>C
t_{max} (h)	3.6 ± 0.31	3.30 ± 0.15	5.4 ± 0.64	A=B<C
$T_{0.5\,(elm)}$ (h)	12.90 ± 1.95	5.50 ± 0.27	3.74± 0.31	A>B>C
K (h^{-1})	0.053± 0.01	0.12 ± 0.01	0.18± 0.02	B<A<C
$T_{0.5\,(Abs.)}$ h	1.52 ± 0.13	1.70 ± 0.15	0.98± 0.10	A<B>C
K_a (h^{-1})	0.45 ± 0.05	0.40 ± 0.03	0.18± 0.02	A=B>C
MRT (h)	14.43 ± 1.83	8.67 ± 0.38	6.78± 0.80	A>B>C
AUC $_{0\text{-}24h}$ (μg·h/mL)	94.25 ± 8.50	55.84 ± 4.51	27.26± 1.92	A>B >C
$AUC_{0\text{-}8}$ (μg·h/mL)	137.1 ± 21.11	56.65 ± 5.61	30.93± 3.36	A>B>C
$AUMC_{0\text{-}24h}$ (μg·h^2/mL)	950.5 ± 28.15	364.88 ±48	210.87±12.36	A>B>C
$AUMC_{0\text{-}8}$(μg·h^2/mL)	1978 ±124.5	384.33 ±49	250.92±14.97	A>B>C
V_d (L)	3.57 ± 0.51	8.55 ± 0.38	2.58± 0.21	A<B<C
Cl_T (m1/min)	3.19 ± 0.43	7.98 ± 0.26	17.93± 3.64	A<B<C
F_R (%)	443.25	183.155	–	–

[a] Mean (± SD, n=6)

[b] C_{max} (the maximum plasma concentration), t_{max} (time to reach C_{max}), $t_{0.5\,(elim)}$ (elimination half-life), K (elimination rate constant), $t_{0.5\,(Abs.)}$ (absorption half-life), K_a (absorption rate constant), $AUC_{0\text{-}24h}$ (area under the plasma concentration time curve from zero time to 24 h), $AUC_{0\text{-}8}$ (area under the plasma concentration time curve from zero time to infinity), $AUMC_{0\text{-}24h}$ (area under first time curve from zero time to 24 h),$AUMC_{0\text{-}8}$ (area under first time curve from zero time to infinity), Vd (volume of distribution), Cl_T (total body clearance), F_R (relative bioavailability of the synthesized compounds to INH)

[c] Statistical analysis was carried out using ANOVA ($p< 0.05$); A=B for means with no significant difference ; A<B or A>B for means showing significant difference.

tives for treatment of tuberculosis infections in order to overcome the resistance developed with INH.

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