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Synthesis And Chemical Stability Of Indolizine Derivatives Of Antihypertensive And Antidiabetic Agents.

Basavaraj M[1]*, Giles D[2], Das Ak[2], and Ganesh S Andhale[2].

[1]Department of Pharmaceutical Chemistry, Vivekananda College of Pharmacy, Rajajinagar, Bengaluru-55, Karnataka, India.
[2]Department of Pharmaceutical Chemistry, Acharya & BM Reddy College of Pharmacy, Bengaluru-107, Karnataka, India.

ABSTRACT

We have synthesized 1,3 substituted indolizines by reacting pyridine, phenacyl bromide and ethylpropiolate in presence of triethylamine, in which the key step is the formation of *N*-acyl pyridinium salt which could participate in a [3+2] intramolecular cycloaddition reaction with ethylpropiolate to form an unstable intermediate which instantly facilitates aromatization to form substitutedindolizine. The formed indolizine reacted with hydralazine and metformin to form the derivatives **(2a-f**). And also we synthesized 1-substituted indolizine, this substituted indolizine formylated to get formylated indolizineand reacted with hydralazine and metformin to form the derivatives (**1a-b**). All the synthesized derivatives were characterized by IR, ^{1}H NMR and Mass spectral data. The synthesized derivatives were studied for chemical hydrolysis at pH 1.2, 6.8, 7.4, and all the derivatives were found be stable at 1.2 pH and partially hydrolyzed at 6.8 and maximum hydrolysis occurred at 7.4 pH. Among the synthesized prodrugs **2b**, **2f** were found to be best among the series.

Keywords: cycloaddition, chemical stability, indolizine, hydrolysis

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Corresponding author

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INTRODUCTION

Hypertension is one of the most life threatening health problems in the modern world. In 2000, the figure of total number of adults with hypertension was 972 million and this may rise by about 60% to a total of 1.56 billion by 2025[1].

Hydralazine is a vasodilator which is used for the treatment of malignant hypertension and hypertensive emergencies, in conjunction with other antihypertensive drugs. Oral administration results in good absorption, extensive hepatic first pass metabolism greatly limited its bioavailability[2,3].

Metformin is a synthetic antidiabetic drug widely used to treat type 2 diabetes. It is protonated under physiological conditions and therefore slowly and incompletely absorbed from the upper intestine after oral administration. Together with a rapid kidney excretion, metformin suffers from the poor bioavailability and causes uncomfortable gastrointestinal side effects at effective doses[4].The biguanides are a class of agent that counteract hyperglycemia primarily through suppression of glucose production in the liver via AMP-activated protein kinase. In skeletal muscle, specifically, insulin stimulates glucose uptake, protein synthesis, and glycogen synthesis and inhibits protein degradation and glycogenolysis. Skeletal muscle is responsible for approximately 75% of insulin-stimulated glucose uptake by the entire body, so in addition to suppressing hepatic glucose production, biguanides also increase insulin sensitivity and enhance glucose uptake in skeletal muscle through AMP mediated protein kinase activation[5].

A typical solution for the above mentioned problems is to use the prodrug approach, in which an applicable bioreversible promoiety is attached to a parent drug. These pharmacologically inactive derivatives require a chemical and/or enzymatic degradation after the delivery to release the parent drug.

Prodrugs are prepared to alter the drug pharmacokinetics, improve stability and solubility, decrease toxicity, increase specificity, and increase duration of the pharmacological effect of the drug. By altering pharmacokinetics the drug bioavailability is increased by increasing absorption, distribution, biotransformation, and excretion of the drug. Limited intestinal absorption, distribution, fast metabolism, and toxicity are some of the causes of failure of drug candidates during drug development[6].

Therefore, it rekindled our curiosity to synthesize various Indolizine carboxylic ester derivatives of antihypertensive and antidiabetic as prodrugs and evaluate them for chemical stability.

MATERIALS AND METHODS

Hydralazine and Metformin obtained as gift samples from Strides Arcolab Bangalore (India).Chemicals used for the synthesis, were purchased from Spectrochem. Solvents were re-distilled before use. Characterization of synthesized derivatives including intermediates, were carried outby FTIR (Bruker spectrophotometer) and, NMR (Bruker-400 spectrometer). Chemical shifts were measured in δ ppm using TMS as internal standard. Mass spectra were recorded on AgilentLC-MS spectrometer. TLC were performed on pre-coated silica gel plates (Merck 60 F_{254}) to check the progress of reaction as well as purity of the synthesized molecules. Melting point was checked by open capillary tube method and reported uncorrected.

General procedure for derivatives (1a, 1b)

An equimolar quantity of (2b, 1mmol) and hydralazine/metformin (1mmol) and add 20 mL of ethanol few drops of acetic acid refluxed and reaction was monitored with TLC after completion of reaction the reaction mixture poured into ice cold water and ppt formed is filtered and dried, purified with ethanol and chloroform mixture.

3-2(pthalazin-1-yl)hydrazine)methyl)indolizine-1-carbonitrile (1a): yellow solid, mp: 190°C. IR (cm^{-1}): 3280.32 (NH, str), 3106.76 (Ar. CH, str), 2980.16 (Ali. CH, str), 2292.95 (CN, str), 1685.12 (C=N). ^{1}H NMR (400 MHZ, $CDCl_3$): δ = 8.778 (s, 1H, CH), 8.490-8.564 (m, 4H, Ar-H), 8.237-8.257 (d, 1H, Ar-H), 7.282 (s, 1H, CH), 7.279 (s, 1H, NH), 7.172-7.265 (m, 4H, Ar-H). MS: m/z 312 (M).

3-(2-(1-cyanoindolizn-3yl)methylene)hydrazinyl)-2-imino-*N,N*-dimethylacetimidamide (1b): pale yellow solid, mp: 135°C. IR (cm^{-1}): 3212.61 (NH, str), 3201.94 (NH, str), 3103.74 (Ar. CH, str), 2985.27 (Ali. CH, str), 2291.23 (CN, str), 1680.12 (C=N). ^{1}H NMR (400 MHZ, $CDCl_3$): δ = 8.778 (s, 1H, CH), 7.431 (s, 1H, CH), 7.111-7.262 (m, 4H, Ar-H), 4.049 (s, 1H, NH), 3.279 (s, 1H, NH), 2.499 (s, 1H, NH), 1.978 (s, 6H, $2CH_3$). MS: (m/z) 281 (M^+).

General procedure for substituted indolizine:

Substituted Phenacyl bromide (1 mmol), pyridine (1.2 mmol), ethylpropiolate (1.4 mmol), triethylamine (0.2 mL) and toluene 10 mL refluxed in RBF for 6 hchecked with TLC for completion of reaction then extracted with chloroform and methanol, washed with water and the organic layer collected, solvent is removed under reduced pressure and the residueobtained is purified with column chromatography using Ethyl acetate hexane as eluent (2:8).

General procedure for the preparation of derivatives (2a):

Substituted indolizine (1 mmol) and the hydralazine (2.5) mmol and sodium methoxide 5mmol at 50° C in toluene refluxed, completion of reaction monitored by TLC.When the reaction was completed a solid formed filtered and purified with ethanol.

3-benzoyl-*N*-(pthalazin-1yl)indolizine-1-carbohydrazide(2a): mp: 206-210°C. IR (cm-1): 3462.56 (NH, str), 3076.87 (Ar. CH, str), 2918.73 (Ali. CH, str), 1694.16 (C=O, str), 1524.45 (CN, str). ^{1}H NMR (500 MHZ, DMSO-d6): δ = 9.973 (s, 1H, CONH), 8.851 (s, 1H, CH), 8.392-8.397 (d, 1H, Ar-H), 7.812-7.997 (m, 4H, Ar-H), 7.444-7.602 (m, 6H, Ar-H), 7.264 (s, 1H, NH), 7.086-7.116 (m, 3H, Ar-H). MS: (m/z) 407(M+).

3-(3-chlorobenzoyl)-*N*-Pthalizin-1-yl)indolizine-1-carbohydrazide(2b): mp: 216-220°C IR (cm^{-1}) 3210.12 (NH, str), 3172.85 (Ar. CH, str), 2918.73 (Ali. CH, str), 1690.76 (C=O, str), 1611.32 (CN, str). ^{1}H NMR (500 MHZ, DMSO-d_6): δ =9.970 (s, 1H, CONH), 8.849 (s, 1H, CH), 8.391-8.413 (d, 1H, CH), 7.811-7.832 (m, 1H, CH), 7.441-7.601 (m, 6H, Ar-H), 7.081-7.100 (m, 5H, Ar-H), 7.251 (s, 1H, NH). MS: (m/z) 441(M^+).

3-(3-bromobenzoyl)-*N*-Pthalizin-1-yl)indolizine-1-carbohydrazide(2c): mp:220-225°C IR (cm-1) 3231.99 (NH, str), 2999.73 (CH, Ali str),1687.18 (C=O, str), 1601.45(CN, str). ^{1}H NMR (500 MHZ, DMSO-d_6): δ= 9.975 (s, 1H, CONH), 8.847 (s, 1H, CH), 8.395-8.416 (d, 1H, CH), 7.812-7.834 (m, 1H, CH), 7.446-7.602 (m, 6H, Ar-H), 7.087-7.104 (m, 5H, Ar-H), 7.473 (s, 1H, NH). MS: (m/z) 485(M^+).

3-benzoyl-*N*-(*N*-(*N,N*-dimethylcarbamimidoyl)indolizine-1-carboxamide(2d):mp:160-165°C. IR (cm^{-1}): 3227.97 (NH, str), 3207.97 (NH, str), 3127.97 (Ar. CH, str), 2959.23 (Ali. CH, str), 1611.23 (C=O, str), 1545.67 (CN, str). ^{1}H NMR (500 MHZ, DMSO-d6): δ= 9.472 (s,1H, CONH), 8.642 (s, 1H, CH), 7.609-7.643 (m, 4H, Ar-H), 7.261-7.492 (m, 5H, Ar-H), 7.261(s, 1H, NH), 4.583 (s, 1H, NH), 4.384 (s,1H, NH), 1.575 (s, 6H, 2CH3). MS: (m/z) 375(M+).

3-(3-chlorobenzoyl)-*N*-(*N*-(*N,N*-dimethylcarbamimidoyl)carbamidoyl)indolizine-1-carboxamide(2e): mp:170-175°C. IR (cm^{-1}): 3212.23(NH, str), 3210.58 (NH, str), 3105.23 (Ar. CH, str), 2999.17 (Ali. CH, str), 1664.65 (C=O, str), 1591.59 (CN, str). ^{1}H NMR (500 MHZ, DMSO-d6): δ= 9.462 (s,1H, CONH), 8.638 (s, 1H, CH), 7.605-7.641 (m, 4H, Ar-H), 7.251-7.482 (m, 4H, Ar-H), 7.251(s, 1H, NH), 4.573 (s, 1H, NH), 4.374 (s,1H, NH), 1.565 (s, 6H, 2CH3). MS: (m/z) 410(M^+).

3-(3-bromobenzoyl)-*N*-(*N*-(*N,N*-dimethylcarbamidmidoyl)carbamidoyl)indolizine-1-carboxamide(2f): mp:180-184°C. IR (cm^{-1}): 3222.45 (NH, str), 3215.57 (NH, str), 3112.12 (Ar. CH, str), 2976.21 (Ali. CH, str), 1634.21(C=O, str), 1581.14 (CN, str). ^{1}H NMR (500 MHZ, DMSO-d6): δ= 9.469 (s,1H, CONH), 8.628 (s, 1H, CH), 7.601-7.637 (m, 4H, Ar-H), 7.255-7.487 (m, 4H, Ar-H), 7.241 (s, 1H, NH), 4.575 (s, 1H, NH), 4.384 (s,1H, NH), 1.567 (s, 6H, 2CH3). MS: (m/z) 454(M^+).

Hydrolysis study

Hydrolytic behavior of synthesized prodrugs was studied in Simulated Gastric Fluid (pH 1.2; USP 1970); Simulated Intestinal Fluid (pH 6.8); Simulated Plasma Fluid (pH 7.4; USP 1970). The hydrolysis was performed by using USP-II paddle apparatus at a rotational speed of 50 rpm. 900 ml solution of pH 1.2, 6.8 and 7.4 were used as dissolution media and maintained at 37±1°C. 1 ml of the hydrolysis medium was taken out at

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zero minute and every 15 min. for 120 min. 1 ml of the pH solution was added to the dissolution vessel. The sample withdrawn was subjected for HPLC analysis using Phenomenex Luna C18 column (250 mm x 4.6 mm id, 5 µm particle size), mobile phase acetonitrile: water 70:30. Flow rate of mobile phase was kept at 1 mL/min at pressure 120-135 psi and UV detector (SPD-20A with D2 lamp) was used.

Under experimental conditions, the prodrug hydrolyzed to release the parent drugs as evident by HPLC analysis. Negligible hydrolysis was observed in acidic buffer (pH 1.2).In other systems, (at constant pH 6.8 and 7.4) the prodrug hydrolyzed and the reaction displayed 1st order kinetics. As the kobs was fairly constant and a straight line plot could be obtained by plotting log concentration of residual prodrug v/s time[13,14].
The comparative study of rate of hydrolysis is shown as follows.

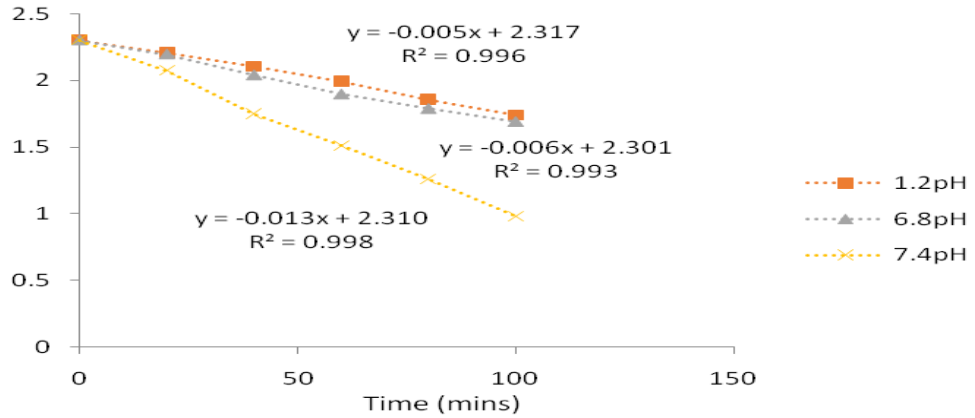

Figure 1: The hydrolysis rate of compound 1a at different pH values.

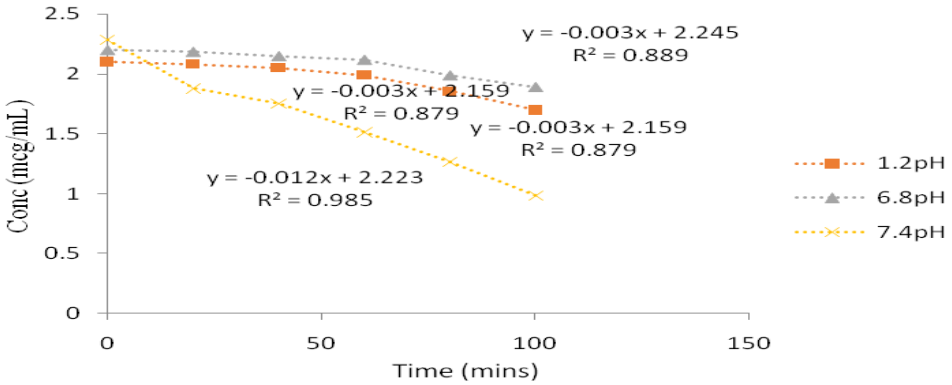

Figure 2: The hydrolysis rate of compound 1b at different pH values.

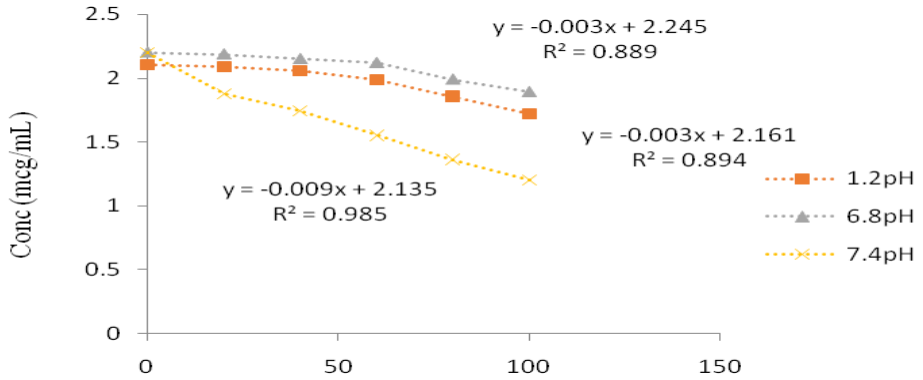

Figure 3: The hydrolysis rate of compound 2a at different pH values.

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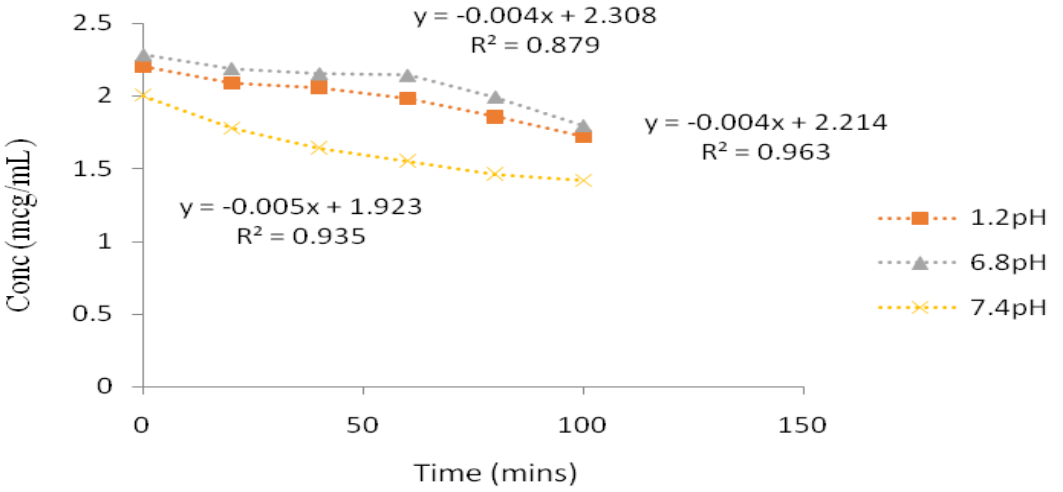

Figure 4: The hydrolysis rate of compound 2b at different pH values.

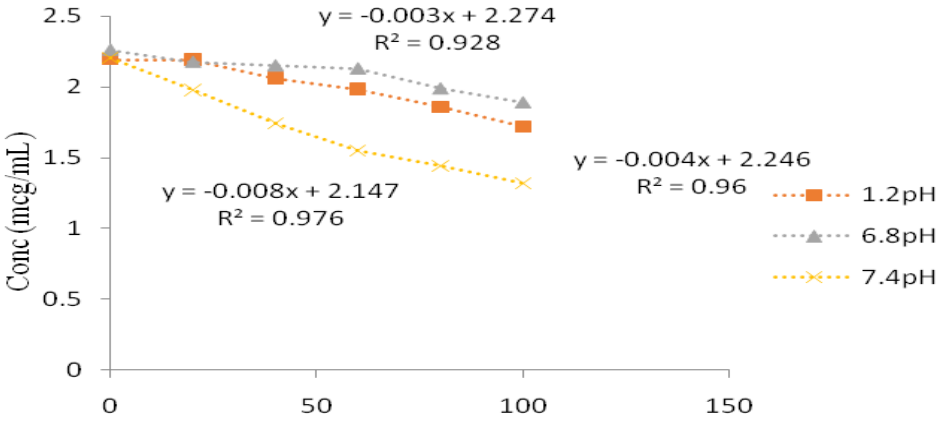

Figure 5: The hydrolysis rate of compound 2c at different pH values

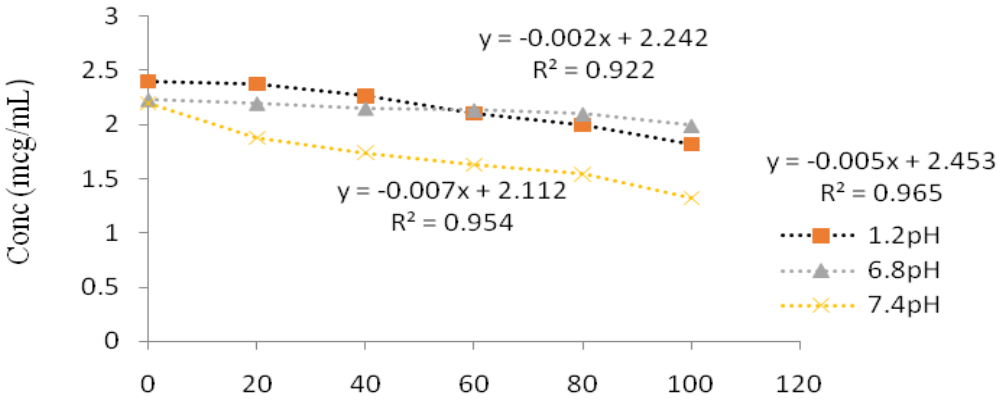

Figure 6: The hydrolysis rate of compound 2d at different pH values

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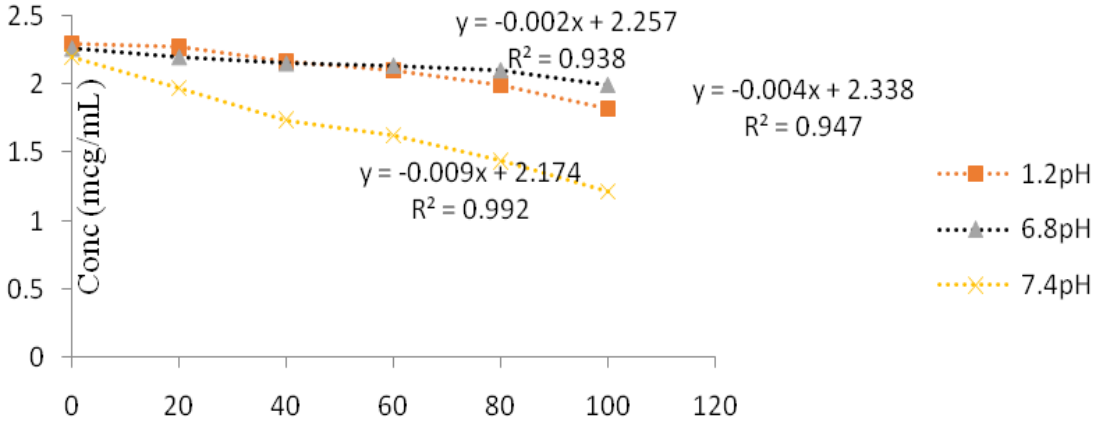

Figure 7: The hydrolysis rate of compound 2e at different pH values

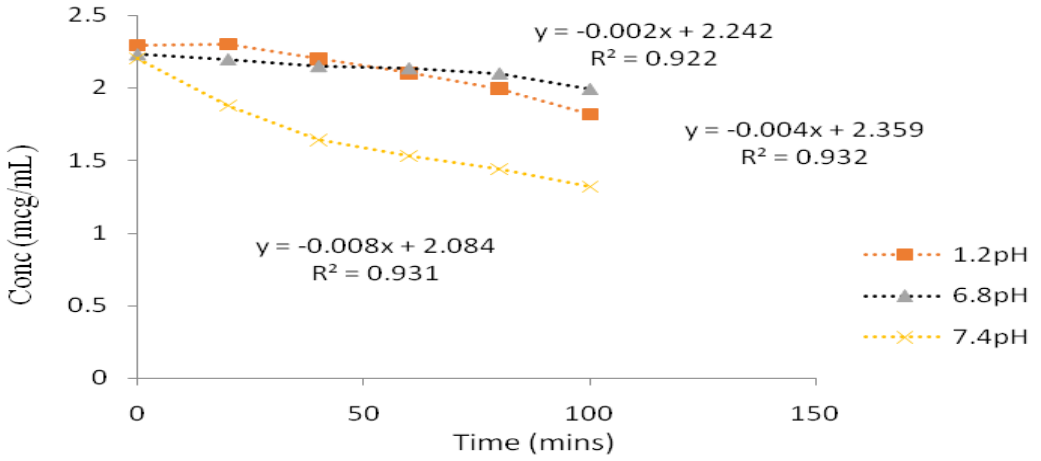

Figure 8: The hydrolysis rate of compound 2f at different pH values

RESULTS AND DISCUSSION

Indolizine 1- carbonitrile was synthesized by using pyridine and chloroacetic acid in presence of ethyl acetate as solvent to form pyridinium salt which was recrystallized from methanol. This salt was reacted with acrylonitrile and using manganese dioxide as oxidant to form the indolizine 1-carbonitrile.Formylation of Indolizine 1-carbonitrile to form 3- formyl indolizine 1-carbonitrile. This was reacted with hydralazine and metformin to form the derivatives. Substituted indolizines have been prepared by treating with ethylpropiolate, substituted phenacylbromide in presence of triethylamine and toluene as solvent. The products were purified by column chromatography using silica gel hexane-ethyl acetate as solvent and the yield was found to be 60-70%. This was treated with hydralazine, metformin to form the corresponding derivatives (**2a-f).** All the reactions was monitored using pre coated TLC plates.The absence of TLC spots for starting material and appearance of single new spot at different R_f Value ensured completion of reaction. TLC plates were visualized either by iodine vapors or by viewing in UV-visible chamber. The structures of synthesized compounds are confirmed by IR, ^{1}H NMR and Mass spectral data**.** Formation of compound (**1a-b)** was confirmed by the presence of imine stretching peaks at 1685.12 cm^{-1} and singlet in the range of δ 8.778-8.672 ppm for imine. The structure of substituted indolizine (**2a-f**) was confirmed by the presence of carbonyl sharp absorption band at 1694.16 cm^{-1} in its IR spectrum. In the ^{1}H NMR spectra, all protons were seen according to the expected integral values. The aromatic protons appeared in the range of δ 7.114 - 8.642 ppm. The ^{1}H NMR spectrum also supports the scheme of synthesis by the absence of peak appeared at δ value 4.114-3.125 ppm which corresponds to $COOC_2H_5$ functional group indicating that the $COOC_2H_5$ group was involved in the reaction. It was converted to amide by formation of a new peak at δ value 9.973-9.472 which

confirms the formation of prodrugs. The mass spectra of compounds gave molecular ions of medium intensity and the base peak usually belonged to the corresponding ions.

The hydrolysis of the synthesized prodrugs **1a,-b**, **2a-f** were studied in aqueous buffer solutions of pH 1.2 (non enzymatic Simulated Gastric Fluid, SGF), pH 6.8 (non enzymatic Simulated Intestinal Fluid) and pH 7.4 (Simulated Plasma Fluid) at 37±5°C using HPLC. As a general pattern, the synthesized prodrugs showed relative stability in the aqueous solutions and the degradation rates at pH 7.4 are slightly accelerated than those observed in SGF of pH 1.2 and SIF of pH 6.8 except and **1a**, **2e,** showed the more hydrolysis at pH 7.4 compared to pH 1.2 and 6.8. Prodrugs **2b, 2f** showed intermediate hydrolysis when compared with **1b, 2a, 2c, 2d.** However, it hydrolyzed to different extent at basic pH in other systems. The present study reveals the importance of exploring prodrugs to obtain compounds of pharmaceutical interest.

Another advantage of prodrugs is their stability in simulated gastric fluid (SGF) (pH 1.2), which facilitate their stomach absorption in a less ionized form in the case of oral administration.

Conclusion

The method using 1, 3-dipolar cycloaddition of pyridinium *N*-methylides with electron-deficient alkenes attracted our attention due to its flexibility and convenience. When electron-deficient alkenes were used in presence of oxidant promoted 1, 3 dipolar cycloaddition for the preparation of indolizine**,** Formylation of indolizine is carried and reacted with hydralazine, metformin to form the prodrugs **1a,** and **1b**.It is proposed that the *N*-alkylpyridinium salt, generated in situ from condensation of phenacyl bromide and pyridine will get converted into the 1,3-dipole species under theinfluence of base and cycloaddition to ethyl propiolate to form an unstable intermediate, which instantly facilitates aromatization to afford comprising substituted indolizine.

Absorption bands obtained in IR and NMR spectrum confirmed the formation of amide linkage between hydralazine and metformin. Preliminary kinetic study for compounds **1a-b, 2a-f** revealed that compounds were chemically stable to a great extent at pH 1.2. This result implies chemical stability and suggests that the compound passes unhydrolyzed through stomach after oral administration. The compound also showed enough stability at pH 7.4 to be absorbed intact from the intestine. Hydrolysis pattern of the best prodrug **2b**, **2f** indicate the release the active drugs for longer period of time. On the basis of the results obtained in this work.

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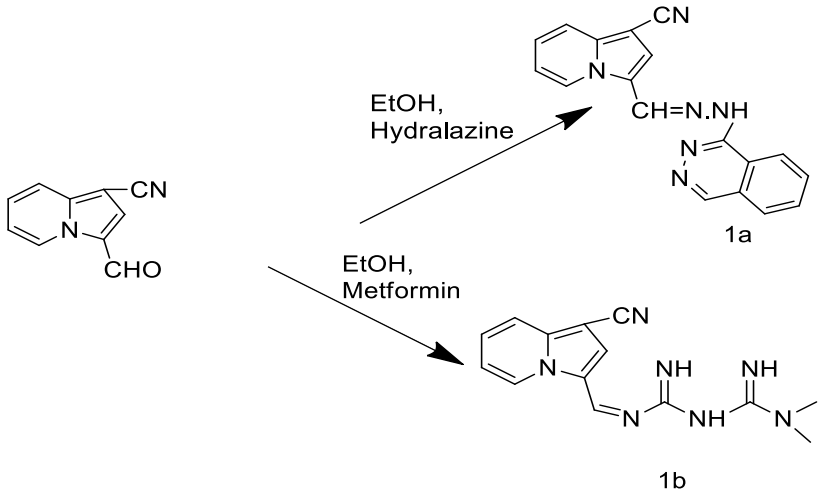

Scheme I: Synthesis of compounds 1a-b.

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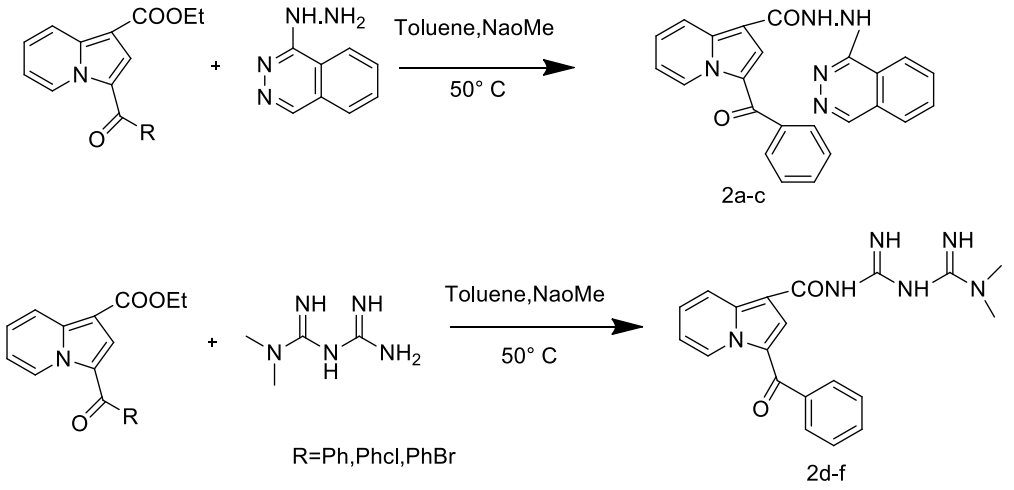

Scheme II: Synthesis of compounds 2a-f.

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