Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2011, 3 (6):224-234
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis, Antimicrobial Evaluation and Electrochemical Studies of some Novel Isoxazole Derivatives

M. T. Shreenivas[1], B. E. Kumara Swamy[1]*, J. G. Manjunatha[1], Umesh Chandra[1], G. R. Srinivasa[2], B. S. Sherigara

[1]*Department of P.G. Studies and Research in Industrial Chemistry, Kuvempu University, Shankaraghatta, Shimoga, Karnataka, India*
[2]*Alkem Laboratories, Peenya Industrial Area, Bangalore, Karnataka (S), India*

ABSTRACT

In the present research work a series of isoxazole derivatives have been synthesized. The isoxazole derivatives are prepared starting from ethylacetoacetate with triethyl orthoformate followed by cyclization and hydrolysis of ester to form 5-Methylisoxazole-4-carboxylic acid. Further the acid is converted into acid chloride followed by condensation leads to a new series of 5-methyl-N-(substituted aryl) isoxazole-4- carboxamides (9 a-f, 13 a-c). The structures of newly synthesized compounds are confirmed on the basis of IR, 1H NMR of spectral data. The newly synthesized title compounds were screened for their in vitro antimicrobial activity. The compounds N-(2-chloro-5-nitrophenyl)-5-methylisoxazole-4-carboxamide (9 e) and N-(4-cyano-3-(trifluoro-methyl)-phenyl)-5-methylisoxazole-4-carbox-amide (9 f) are shown good antibacterial and anti fungal activity. The electrochemical reduction was carried out by using cyclic voltammetric technique and the effects of scan rate, sulphuric acid concentration and substitutents were studied. The overall electrode process was diffusion-controlled.

INTRODUCTION

The biological activity of substituted isoxazoles [1] has made them a focus of medicinal chemistry over the years. The extensive investigation of biological activity of organic compounds has revealed the presence of several active compounds in the isoxazole series. Biologically the most important compound of naturally occurring isoxazole series is the antibiotic cycloserine, which is 4-amino-3-isoxazolidine possessing antitubercular, antibacterial and also antileprotic properties. The therapeutic potential of isoxazoline derivatives is further evident from their antibacterial[2,3] antifungal[4,5], analgesic, antiviral [6], anti-inflammatory[7], anti convulsant, COX-2 enzyme inhibitory activit[8], anticancer [9], anti-HIV[10], $GABA_A$ antagonist activity[11] and anti-HIV activites[12]. The Biphenyl ethers are intermediates for the many drugs and play a vital role in synthesis and biological activities. The importances of these are revealed by the drugs such as Nimesulide, Levothyroxin, Fenoprofen,

etc. The combinations of different substructures are the one of the approach to synthesize potential biologically active compounds from known active compounds.

Glassy carbon is widely used as an electrode material in electrochemistry for the detection of many electroactive substances. A number of carbon electrodes have been introduced into voltammetric studies [13, 14]. These electrodes have a very wide anodic as well as cathodic potential region, both in aqueous and non-aqueous solvents. Carbon paste made of graphite powder and mineral solvent was one of the earliest inert electrodes introduced into the electrochemistry [15]. The vitreous or glassy carbon electrode introduced into electrochemistry is the most widely used carbon electrode material today [16, 17]. Its high mechanical stability, low porosity, inertness over a wide potential region and good conductivity and reproducibility are some of the reasons for its very wide applications.

The approach to the preparation of potential biologically active compounds and in continuation of our ongoing studies on novel biologically active molecules, we have designed and synthesized some novel isoxazole derivatives as described in **Scheme-1.** The synthesized compounds were evaluated for its antibacterial activities and electrochemical properties are studied.

MATERIALS AND METHODS

The Ethylacetoacetate, Acetic anhydride, Triethylorthoformate, 50% Aq. Hydroxylamine solution and oxalyl chloride were obtained as LR grade from S.D. fine chemicals. The 2-phenoxy aniline, 4-nitro-2-phenoxy aniline and 4,6-dibromo-2-phenoxy aniline were prepared according to the literature[18]. The solvents and are other chemicals were obtained from commercial sources. The column chromatography was performed on silica gel (60-120 mesh) using Hexane and Ethyl acetate and TLC with silica gel GF254 plates (Merck). The spots were detected in an iodine chamber and under UV light. The LCMS and GCMS were recorded in Thermo Fennigen LCQ Advantage Max LCMS and Trace GC Ultra respectively. ^{1}H and ^{13}C NMR spectra were recorded on 400-MHz Bruker spectrometer from Punjab University and IISc, Bangalore respectively. Infrared spectra were recorded on a Thermo Scientific Nicolet 6700 FT-IR spectrometer. Melting points were recorded (uncorrected) on Buchi Melting Point B-545 instrument. Chemical shifts are expressed in δ (ppm) relative to TMS as the internal standard and coupling constants (J) are reported in hertz (Hz). Reactions were carried out in oven dried glassware under nitrogen. Evaporations were conducted under reduced pressure at temperature less than 45°C unless otherwise noted. All other reagents were used without further purification.

3.1. Preparation of 5-methylisoxazole-4-carboxylic acid

(a) Preparation of Ethyl ethoxymethyleneacetoacetate (2)

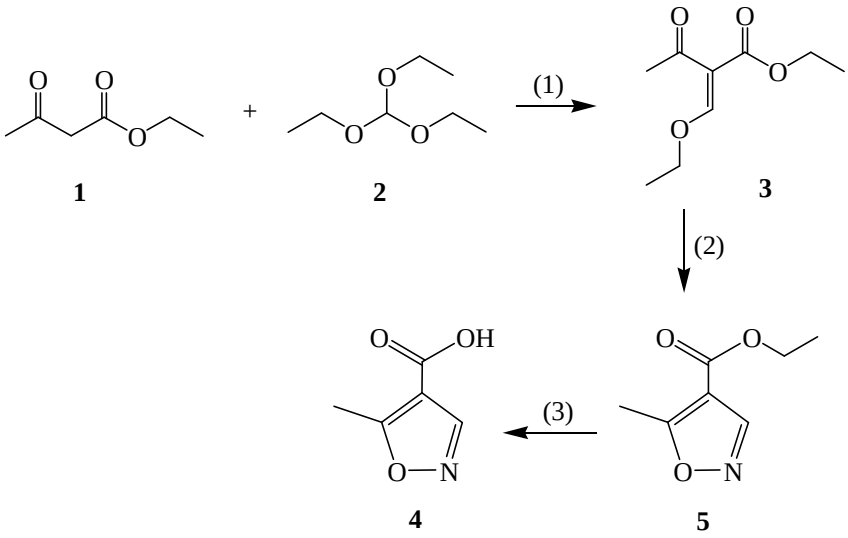
A mixture of ethylacetoacetate (100.00 g, 0.768 mol), acetic anhydride (156.89 g, 1.536 mol) and triethylorthoformate (138.93 g, 0.937 mol) were heated to 135°C. The byproducts formed were distilled off simultaneously. After attaining a temperature of 135°C, the reaction mixture was stirred for 1 h. The left over byproducts was distilled out by applying 50 mm vacuum and was cooled to 20-30°C to obtain 114.0 g of Ethyl ethoxymethyl-eneacetoiacetate as blackish thick oil. The oily material is used for the next step without further purification.

(b) Preparation of Ethyl-5-methylisoxazole-4-carboxylate (3)

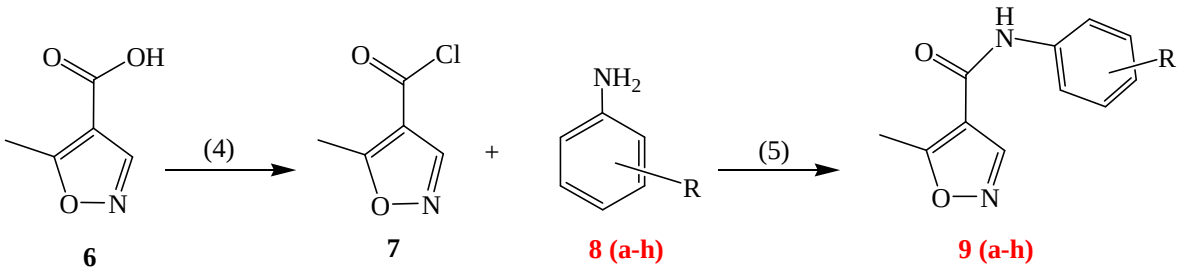
Dissolved Ethyl ethoxymethyleneacetoacetate (110.00 g, 0.59 mol) in 330 mL of methanol and cooled to -5°C and 50% aqueous hydroxyl amine (39.03 g, 0.59 mol on anhydrous basis) was added drop wise over a period of 1 h at -5 to 0°C and stirred for 30 min. Then reaction mixture was allowed to attain temperature of 20 to 30°C and refluxed for 1 h. After completion of

reaction the solvent was removed under reduced pressure. To the residue 500 mL of n-Hexane was added and stirred for 30 min at room temperature. Once again 100 mL of saturated sodium bicarbonate solution followed by 400 mL of water was added and stirred for 30 min. The organic layer was separated and the aqueous layer was re-extracted with n-hexane (2 X 200 mL). The combined organic layer was washed with water (2x250 mL). After removal of the solvent 72.5 g (79%) of Ethyl-5-methylisoxazole-4-carboxylate was obtained as yellowish thick oil.

Scheme-1

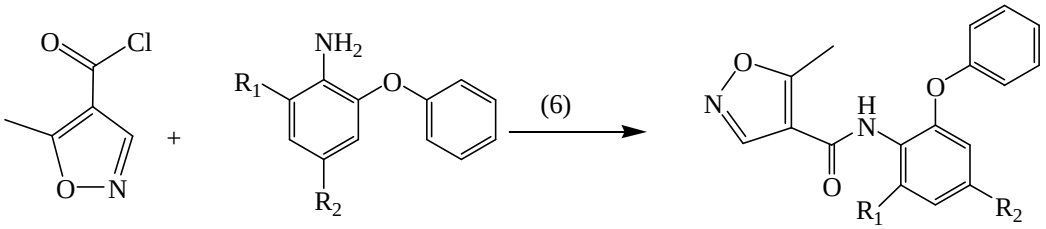


Reagents: (1) Ac_2O, RT to 135°C, 1 h,135°C (2) MeOH, RT to 5°C, NH_2OH, H_2O, 1 h, -5-10°C, 30 min. (3) H_2SO_4, H_2O, 16 h, reflux



Reagents: (4) Thionyl Chloride, Chloroform, 10°C-Reflux, 1 h. (5) Acetonitrile, RT, 18 h.

R	Ortho position	Meta position	Para position
a	H	H	NO_2
b	NO_2	H	NO_2
c	H	COOH	OH
d	H	H	CN
e	H	CF_3	CN
f	NO_2, Cl	H	H
g	H	H	OCH_3
h	H	H	CH_3



7 **(10, 11,12)** **13 (a-c)**

Reagents: (6) Acetonitrile, RT, 18 h.

	10	11	12	13		
				a	b	c
R_1	H	H	Br	H	H	Br
R_2	H	NO_2	Br	H	NO_2	Br

(c) Preparation of 5-MethyIisoxazole-4-carboxylic acid (4)

Ethyl-5-methylisoxazole-4-carboxylate 72.58 g (0.47 mol) was refluxed in 20% v/v aqueous sulphuric acid (195 mL) for 16 h and then 75 mL of Toluene was added at 90°C. The reaction mixture was cooled to 25 to 35°C and stirred for 4 h. The crystallized solid product was filtered and washed with toluene (2 x 36 mL) followed by water (2 x 75 mL). Then product was dried under vacuum to get 27.2 g of 5-Methylisoxazole-4-carboxylic acid as an off white solid. m. p. 144-147°C; FTIR (Neat): 1716 (C=O), 1601 (C=N), 3113(-OH); GCMS: *m/z* 127. ^{1}H NMR (400 MHz, DMSO-d6): δ 2.49-2.63 (3H, s, -CH_3) , δ 8.75 (H, s, CH=CH), δ 13.09 (H, s, C00H); GCMS: *m/z* 127.

1.2. Preparation of 5-methylisoxazole-4-carbonyl chloride (5)

5-MethyIisoxazole-4-carboxylic acid (27 g, 0.1855 mol) was dissolved in 250 mL chloroform at 0-5°C with stirring. To a reaction mixture, oxalyl chloride (35.32 g, 0.2782 mol) was added drop wise and then heated to reflux for 1 h. The reaction was monitored by TLC (System-Hexane:EtOAc::7:3). After completion of reaction, the reaction mixture was condensed and added 100 mL of chloroform and solvent was evaporated, it was repeated thrice and the residue is used in next step without any workup and purification.

1.3. General Procedure for preparation of 5-methyl-N-(substituted aryl) isoxazole-4-carboxamides (9 a-f, 13 a-c)

To a stirred solution of amine (1.0 mmol) in acetonitrile (20 mL), was added 5-methylisoxazole-4-carbonyl- chloride (1.5 mmol) drop wise at 0-5°C. The resulting mixture was stirred at room temperature for overnight. The reaction was monitored by TLC (System-Hexane:EtOAc::7:3). After completion of reaction, the reaction mixture was condensed and the residue was washed with diethyl ether to remove non polar impurities. Once again residue was stirred with cold methanol at 5°C for 30 min to get almost pure (>95%) compound. Few compounds were purified through column chromatography using silica gel (60-120) and hexane: ethyl acetate as an eluent to get purest compound.

i) Preparation of 5-methyl-N-(4-nitrophenyl)isoxazole-4-carboxamide (9 a)

To a stirred solution of 4-nitroaniline (2.0 g, 14.4 mmol) in 25 mL acetonitrile, was added 5-methylisoxazole-4-carbonyl chloride (3.16 g, 21.72 mmol) drop wise at room temperature. The resulting mixture was stirred at room temperature for overnight. The reaction was monitored by TLC (System-Hexane:EtOAc::7:3). After completion of reaction, the reaction mixture

(suspension) was filtered and the filtrate was evaporated in a rota vapour under high vacuum. The residue was washed with diethyl ether to remove non polar impurities as well as starting materials. Once again residue was stirred with cold methanol at 5°C for 1-2 h and dried in a vacuum oven. Yield: 2.79 g (52%); m. p. 166-169°C; IR(Neat): 1681 (Amide, C=O), 1591 (-NO_2), 1496 (C=N), 1240 (C-O) Cm-1; ^{1}H NMR (400 MHz, DMSO-d6): δ 2.43-2.46 (3H, s, -CH_3) , δ7.11-7.90 (4H, m, Ar), δ 9.47 (H, s, C=H), δ 10.52 (H, s, -CO-NH).

ii) Preparation of 5-methyl-N-(4-nitro-2-phenoxyphenyl)isoxazole-4-carboxamide (13 b)
To a stirred solution of 4-nitro-2-phenoxyaniline (1.5 g, 6.5 mmol) in 20 mL acetonitrile, was added 5-methylisoxazole-4-carbonyl chloride (1.42 g, 9.7 mmol) drop wise at room temperature. The resulting mixture was stirred at room temperature for overnight. The reaction was monitored by TLC (System-Hexane:EtOAc::7:3). After completion of reaction, the reaction mixture (suspension) was filtered and the filtrate was evaporated in a rota vapour under high vacuum. The residue was washed with diethyl ether to remove non polar impurities as well as starting materials. Once again residue was stirred with cold methanol at 5°C for 1-2 h and dried in a vacuum oven. Yield: 1.6 g (75%), m. p. 218-220°C; IR (Cm^{-1}, KBr): 3300 (NH), 1684 (Amide, C=O), 1534 (-NO_2 asym str.), 1505(C=N), 1489(C-N), 1478, 1417, 1373, 1329 (-NO2 sym str.), 1239, 1161 (C-C str.), (C-O-C) ; ^{1}H NMR (400 MHz, CDCl3) (ppm): δ 2.77 (3H, s, -CH3) δ 7.10-8.74 (8H, m, Ar), δ 8.41 (H, s, CH), δ 8.30 (H, s, -CO-NH); ^{13}C NMR (400 MHz, $CDCl_3$) (ppm): 173.52-173.55, 159.04, 154.38, 147.39, 145.98, 143.39, 134.43, 130.71, 125.91, 119.52-119.63, 112.04, 111.45-111.49, 12.69-12.71.

The spectral and analytical data isoxazole derivatives are given below.
iii) 5-methyl-N-(2,4-dinitrophenyl)isoxazole-4-carboxamide (9 b): Yield: 1.12 g (47%); m. p. 183-185°C; IR (Neat): 1698 (Amide, C=O), 1598 (-NO_2), 1486 (C=N), 1225 (C-O) Cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d6): δ 2.40-2.43 (3H, s, -CH_3), δ 7.11-7.90 (3H, m, Ar), δ 9.47 (H, s, C=H), δ 10.48 (H, s, -CO-NH).

iv) 5-(5-methylisoxazole-4-carboxamido)-2-hydroxybenzoic acid (9 c): Yield: 1.23 g (36%); m. p. 214-217°C; IR (Neat): 1681 (COOH), 1656 (Amide, C=O), 1431 (C=N), 1234 (C-O), 3100 (-OH) Cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d6): δ 2.48-2.49 (3H, s, -CH_3), δ 7.19-7.76 (3H, m, Ar), δ 9.05-9.06 (H, s, C=H), δ 9.10.19 (H, s, -OH), δ 11.38 (H, s, -CO-NH), δ 13.84 (H, b, -COOH).

v) N-(2-cyanophenyl)-5-methylisoxazole-4-carboxamide (9 d): Yield: 0.89 g (41%); IR (Neat): 2230 (-CN), 1659 (Amide, C=O), 1466 (C=N), 1305 (C-O) Cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d6): δ 2.42-2.44 (3H, s, -CH_3), δ 7.08-7.88 (4H, m, Ar), δ 9.45 (H, s, C=H), δ 10.52 (H, s, -CO-NH).
vi) N-(2-chloro-5-nitrophenyl)-5-methylisoxazole-4-carboxamide (9 e): Yield: 0.78 g (48%); m. p. 135-137°C; IR (Neat): 1673 (Amide, C=O), 1603 (-NO2), 1500 (C=N), 1234 (C-O) Cm-1; ^{1}H NMR (400 MHz, DMSO-d6): δ 2.48-2.49 (3H, s, -CH3), δ 7.50-8.06 (3H, m, Ar), δ 9.04 (H, s, C=H), δ 10.70 (H, s, -CO-NH).

vii) N-(4-cyano-3-(trifluoromethyl)phenyl)-5-methylisoxazole-4-carbox-amide(9f): Yield: 0.80 g (51%); m. p. 114-116°C; IR (Neat): 2236 (-CN), 1690 (Amide, C=O), 1500 (C=N), 1245 (C-O) Cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d6): δ 2.43-2.46 (3H, s, -CH_3), δ 7.71-7.90 (3H, m, Ar), δ 9.40 (H, s, C=H), δ 10.50 (H, s, -CO-NH).

viii) N-(4-methoxyphenyl)-5-methylisoxazole-4-carboxamide (9 g): Yield: 1.8 g (42%); IR(Neat): 1681 (Amide, C=O), 1591 (-NO_2), 1496 (C=N), 1240 (C-O) Cm^{-1}; ^1H NMR (400 MHz, DMSO-d6): δ 2.43-2.46 (3H, s, -CH_3) , δ 3.73 , δ 7.14-7.98 (4H, m, Ar), δ 9.47 (H, s, C=H), δ 10.68 (H, s, -CO-NH).

ix) 5-methyl-N-p-tolylisoxazole-4-carboxamide (9 h): Yield: 1.0 g (35%); IR (Neat): 1673 (Amide, C=O), 1603 (-NO_2), 1500 (C=N), 1234 (C-O) Cm^{-1}; ^1H NMR (400 MHz, DMSO-d6): δ 2.48-2.49 (3H, s, -CH_3), δ 2.35 (3H, s, -CH_3) δ 7.08-7.86 (4H, m, Ar), δ 9.04 (H, s, C=H), δ 10.46 (H, s, -CO-NH).

x) 5-methyl-N-(2-phenoxyphenyl)isoxazole-4-carboxamide (13 a): Yield: 1.26 g (53 %); m. p. 114-116°C; IR(Neat): 1680 (Amide, C=O), 1528 (C=N), 1210 (C-O) Cm-1; ^1H NMR (400 MHz, CDCl3): δ 2.70-2.72 (3H, s, -CH_3) δ 7. 01-8.72 (9H, m, Ar), δ 8.40 (H, s, CH), δ 8.31 (H, s, -CO-NH).

xi) N-(2,4-dibromo-6-phenoxyphenyl)-5-methylisoxazole-4-carboxamide (13 c): Yield: 0.63 g (48%); m. p. 169-171°C; IR(Neat): 1682 (Amide, C=O), 1530 (C=N), 1213 (C-O) Cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.71 (3H, s, -CH_3) , δ 7.11-8.82 (7H, m, Ar), δ 8.42 (H, s, CH), δ 8.36 (H, s, -CO-NH).

Table-1. Antimicrobial and Antifungal Data of Synthesized compounds

Compound	Zone of inhibition (in mm)					
	***S. Aureus*[1]**	***B. Subtilis*[1]**	***E. Coli*[1]**	***P. Aeruginosa*[1]**	***C. albicans*[2]**	***A. Niger*[2]**
	100 µg/ mL	**100 µg/ mL**	**100 µg/ mL**	**100 µg/ mL**	**100 µg/ mL**	**100 µg/ mL**
9 a	11	12	12	14	11	6
9 b	12	14	13	16	10	9
9 c	14	16	15	17	11	12
9 d	09	11	12	15	9	8
9 e	15	18	16	15	12	11
9 f	16	18	16	19	13	11
9 g	12	13	13	17	12	13
13 a	12	14	15	17	8	7
13 b	13	16	16	18	11	9
13 c	14	15	16	17	9	10
Standard: Streptomycin	22	23	26	25	-	-
Standard: Griseofulvin	-	-	-	-	16	15
Control: DMF	-	-	-	-	-	-

[1] *Inactive: less than 12 mm, Weakly active: 12-15 mm, Moderately active: 16-18 mm, Highly active: more than 18 mm.*
[2] *Inactive: less than 10 mm, Weakly active: 10-12mm, Moderately active: 12-14 mm, Highly active: more than 14 mm.*

2. Antimicrobial Activity

Compounds **9 (a-f)** and **13 (a-c)** were screened for their in-vitro antibacterial activity against *S.aureus, B. subtilis E. Coli* and *P. Aeruginosa* employing cup-plate method at the concentration of 100µg/ml in nutrient agar media[19, 20] and also for in-vitro antifungal activity against *C. albicans* and *A. Niger* by cup plate method at 100µg/ml. concentration using sabouraud-dextrose agar. DMSO was used as solvent control for antimicrobial activity. Streptomycin and Griesuofulin were used as standard for antibacterial and antifungal activities respectively. The area of inhibition of zone measured in cm. The results are listed in Table-1.

3. Electrochemical Studies

3.1. Instrumentation

The electrochemical experiments were carried out using a model-660 Electrochemical Workstation (CHI660c). All experiments were carried out in a conventional three-electrode system. The electrode system contained a working Glassy Carbon electrode, a platinum wire as counter electrode and saturated calomel electrode as reference electrode.

In a typical cyclic voltammetric experiment of substrate (Isoxazole derivative), reaction mixture consisted of isoxazole derivative solution, DMSO (to dissolve isoxazole derivative), ethyl alcohol (to keep isoxazole derivative in homogeneous phase) and sulphuric acid media was used for the electrolytic reduction. The three electrodes were connected to a computer controlled potentiostat and required potential scan rate, current sensitivity, initial potential and final potential were fixed and the resulting current measured as a function of applied potential.

3.2. Pre-treatment of glassy carbon electrode

Before each measurement, the glassy-carbon surface was polished with alfa alumiina powder (0.3 µ) on a rubbing pad and then rinsed with purified water. The supporting electrolyte media was placed in the cell and several potential sweeps were applied to obtain a low background. The isoxazoline derivative was added and the first potential sweep was registered.

RESULTS AND DISCUSSION

6.1. Voltammetric behavior of Electrochemistry of Isonitro (5-methyl-N-(4-nitrophenyl)isoxazole-4-carboxamide, 9 a)

Figure 1 shows Cyclic voltammogram of isonitro at glassy carbon electrode in the mixture of ethanol, dimethyl sulfoxide and 0.1 M sulfuric acid at scan rate 0.1 V/s. Electrochemical investigation of isonitro was carried out at glassy carbon electrode in the mixture of ethanol, dimethyl sulfoxide and 1 M sulfuric acid at scan rate 0.1 V/s. The potential window was maintained between 0 to -0.8 V. The cyclic voltammogram of isonitro was shows a reduction peak at -0.37 V corresponds to the reduction of nitro group present in the molecule.

6.2. Effect of concentration

Figure 2 shows the graph of cathodic peak current vs concentration of isonitro. As the concentration of isonitro was varied from 0.2 to 1.0 mM the cyclic voltammograms were recorded. The cathodic peak current Ipc obtained was found to increase linearly with increase in concentrations. The plot of Ipc versus concentrations of Isoxazoline shows linearity up to 0.7mM and at higher concentration current remains almost constant[21, 22].

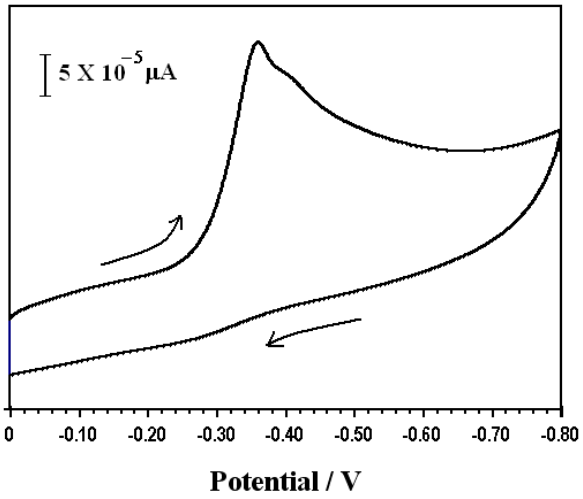


Figure 1. Cyclic voltammogram of isonitro at glassy carbon electrode in 0.1 M sulfuric acid at scan rate 0.1 V/s.

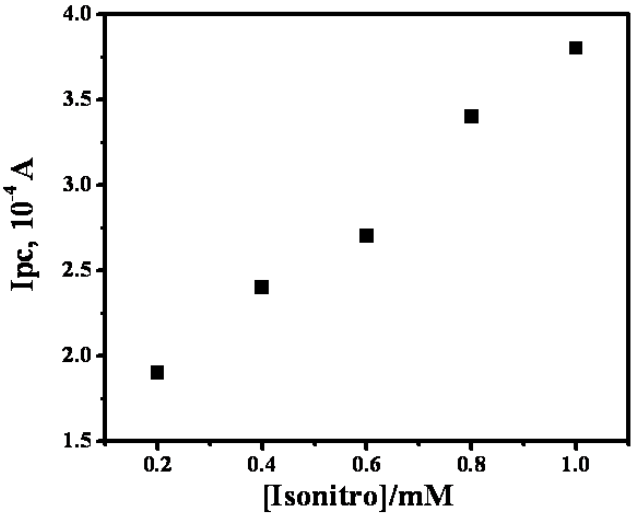


Figure 2. Graph of cathodic peak current vs concentration of isonitro

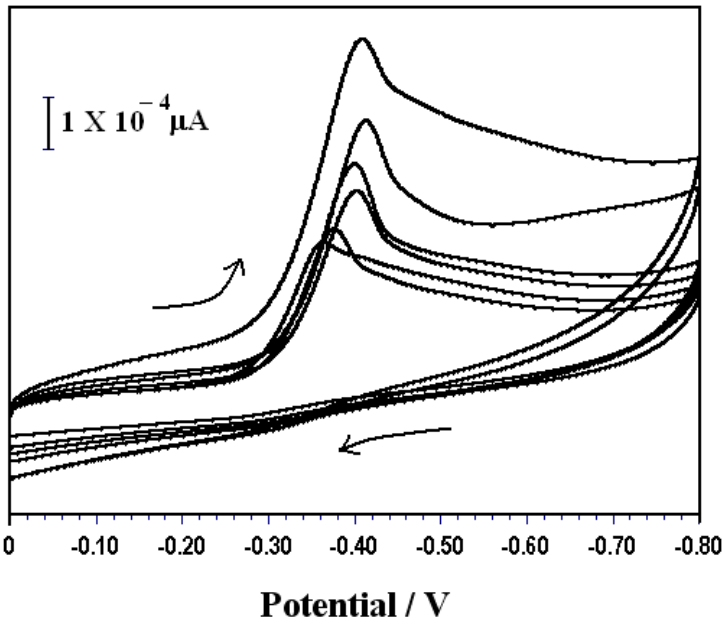


Figure 3. Cyclic voltammograms of isonitro at glassy carbon electrode 0.1 M sulfuric acid at different scan rates

6.3. Effect of scan rate

Figure 3 shows the cyclic voltammograms of isonitro at glassy carbon electrode in the mixture of ethanol, dimethyl sulfoxide and 0.1 M sulfuric acid at different scan rate. The concentration of the isonitro was kept constant at 0.5mM in 0.1 M sulphuric acid and the sweep rate was varied from 100 to 500mVs^{-1}. In all the cases the cathodic peak current was proportional to the square root of the scan rate. Under these conditions the currents were diffusion–controlled [23, 24]. The peak potential was shifted towards more negative values with an increase in the scan rate, indicating that electrochemical process was irreversible. The peak potential shift was higher when the scan rate increased. This means that under these conditions the electrochemical processes are more irreversible.

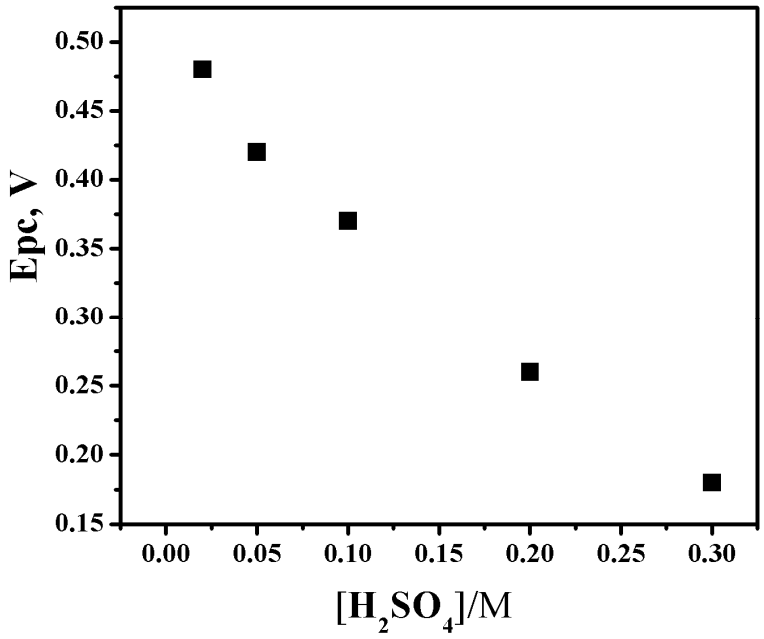


Figure 4. Graph of cathodic peak potential vs sulfuric acid concentration

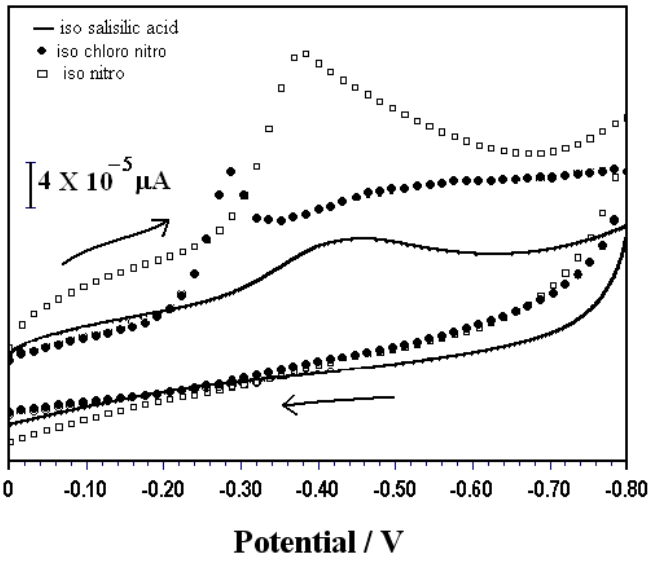


Figure 5. Cyclic voltammograms of (hallow circle line) isonitro, (dotted line) isochloronitro and (solid line) isosalisilic acid in 0.1 M sulfuric acid at glassy carbon electrode at scan rate 0.1 V.

(Isochloronitro: N-(2-chloro-5-nitrophenyl)-5-methylisoxazole-4-carboxamide, 9 e; isosalisilic acid: 5-(5-methylisoxazole-4-carboxamido)-2-hydroxybenzoic acid, 9 c)

6.4. Effect of sulfuric acid concentration

Figure 4 shows the graph of cathodic peak potential vs sulfuric acid concentration.As the concentration of sulphuric acid increases from 0.02 to 0.3 M, the cathodic potential shifts from 0.48 V to 0.18 V. Thereby showing the involvement of protons in the reduction process [23, 25].

6.5. Effect of substituents

Figure 5 shows the cyclic voltammograms of isonitro, isochloronitro and isosalisilic acid in the mixture of ethanol, dimethyl sulfoxide and 0.1 M sulfuric acid at glassy carbon electrode at scan rate 0.1 V. Electron withdrawing group accelerates reduction where as electron-donating group lower it. Accordingly electron releasing substituted isoxazole and nitro substituted isoxazole undergo reduction at – 0.3 mV and – 0.45 mV respectively.

CONCLUSION

All the synthesized isoxazole analogues were tested for their antibacterial and antifungal activities. From the data it revealed that few isoxazoline analogues from the synthesized compounds exhibit moderate to good activity against all the bacteria and fungi tested.

Particularly compounds **9 c, 9 e, 9 f**, **9 g** and **13 b** were active against all the selected gram-negative as well as gram-positive bacteria. Among these compounds **9 e** and **9 f** are found to be more active than others. The compounds **9 a, 9 d** and **13 a** are did not show promised activity against both the bacteria. The synthesized compounds are active against all the selected fungi. Particularly the compounds **9 a, 9 e, 9 f, 9 g** and **13 b** are shown good antifungal activity compared with other compounds. The compounds **9 b, 9 c, 9 d, 13 a** and **13 c** are weekly active to all the fungi.

The outcome of this study shows that there is scope for further studies on N-(2-chloro-5-nitrophenyl)-5-methylisoxazole-4-carboxamide **(9 e)**, N-(4-cyano-3-(trifluoromethyl)-phenyl)-5-methylisoxazole-4-carbox-amide **(9 f)** and 5-methyl-N-p-tolylisoxazole-4-carboxamide **(9 g)** as good antibacterial and anti fungal agents.

Electrochemical behaviour of isonitro shows one reduction cathodic peak at -0.37V and absence of peak in the reverse scan. As the concentration of sulphuric acid increases the peak potential shifts towards positive value indicating easier reduction at higher concentration of sulphuric acid. The cathodic peak current was found to increase linearly with square root of sweep rate and also with concentration of isonitro, suggested that process is diffusion-controlled. The cathodic peak potentials are shifted to more negative values with increase in concentration of isonitro.

REFRENCES

[1] L. Carlsen, D. Do¨pp, H. Do¨pp, F. Duus, H. Hartmann, S. Lang-Fugmann, B. Schulze, R. K Smalley, B .J. Wakefield, In *Houben-Weyl, Methods in Organic Chemistry;* E8a, Schaumann, E., Ed.; Georg Thieme Verlag: Stuttgart, Germany, **1995,** 45
[2] P. Cali, L. Naerum, S. Mukhija and A. Hjelmencrantz, *Bioorg, Med. Chem. Letters.*, **2004,** 14, 5997
[3] M. Sree Rama Murthy, D. Venkata Rao, E. Venkata Rao, *Indian J. Pharma. Sci.*, **1983**, 45, 131 (1983).
[4] J. D. Davenport, A. D. Barry, A. F. Elsasser, Ger. Offen. 2,723,688 (C1. A01N9/28), 22 Dec (1977); US Appl. 695,669, 14 Jun (1976); 43 pp; *Chem. Abstr.*, **1978,** 88, 132015k

[5] D. D. Guy, P. M. Carabates, (Sterling Drug Inc.) U.S. 4,268,678 (C1. 548-247; C07D261/08), 19 May (1981), Appl. 72,134, 04 Sep (1979); 4 pp; *Chem. Abstr.*, **1981,** 95, 203923n
[6] G. Daidone, D. Raffa, B. Maggio, F. Plescia, V. M. C Cutuli, N .G. Mangano, A. Caruso, *Arch. Pharm. Pharm. Med. Chem.*, **1999,** 332, 50
[7] S. Balalaie, A. Sharifi, B. Ahangarian, *Indian J. Heterocyclic Chem.*, 2000, 10, 149
[8] L. D. Nunno, P. Vitale, A. Scilimati, S. Tacconelli, P. Patrignani, *J. Med. Chem.*, **2004,** 47 (20), 4881
[9] V. H. Bhaskar, P. B. Mohite, *J Optoelectron Adv. Mat.*, **2010**, 2, 249
[10] M. Sechi, L. Sannia, F. Carta, M. Palomba, R. Dallocchio, A. Dessi, M. Derudas, Z. Zawahir, N. Neamati, *Antiviral Chem. Chemother.*, **2005,** 16, 41
[11] B. Frolund, A. T. Jorgensen, L. Tagmose, T. B. Stensbol, H. T. Vestergaard, C. Engblom, U. Kristiansen, C. Sanchez, P. Krogsgaard-Larsen, T. Liljefors, *J. Med. Chem.*, **2002,** 45, 2454
[12] B. L. Deng, M. D. Cullen, Z. Zhou, T.L. Hartman, R. W. Buckheit(jr.) , C. Pannecouque, E. Declescq, P.E. Fanwick, M. Cushman, *Bioorg. Med. Chem.*, **2006,** 14, 2366
[13] R. E. Panzer, P. J. Elving, *Electrochemi. Acta*, **1975,** 20, 635
[14] J. P. Randin, *Encyclopedia Electrochem. Elements*, **1976,** 5, 1
[15] R. N. Adams, *Electrochemistry at Solid Electrodes*, (Marcel Dekker, New York) **1969**
[16] H. E. Zittel, F. J. Miller, *Anal. Chem.*, **1965,** 37, 200
[17] W. E. Van Der Linden, J. W. Dieker, *Anal. Chem. Acta*, **1980**, 119, 1
[18] M.T. Shreenivas, B. P Chetan, A. R. Bhat, *J. Pharmaceutical Sci. Tech.*, **2009,** 1, 88
[19] S. Y. Xu, R.L. Bian, X. Chen, *Methadology of Pharmacology Experiment*, Peoples Medical Publishing house, Beijing;, **1994,** 1356
[20] A. W. Bauer, W. M. Kirb, J. C. Shersis, M. Turck, *Am. J. Clin. Pathal.*, **1996,** 45, 493
[21] C. Slifstein, M. Ariel, *J. Electroanal. Chem.*, **1977**, 75, 551
[22] M. N. Hulbert , I. Shain, *Anal. Chem.*, **1970**, 42, 162
[23] S. R. Murali, B.E. Kumara Swamy, B.S. Sherigara, B. Kallurayya. *Bull. Electrochem.*, **2002**, 18, 385
[24] B. Eswarappa, B. S. Sherigara, B. E. Kumara Swamy. *Bull. Electrochem.*, **2004,** 20, 1
[25] A.H.M. Siddalingaiah, S.G. Naik, B.S. Sherigara, B.E. Kumara Swamy. *Bull. Electrochem.*, **2002,** 18, 445