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CHEMISTRY OF NOVEL ISOXAZOLS AND PYRAZOLES CONTAINING 2-ACETYL THIOPHENE- THEIR SYNTHESIS AND ANTIMICROBIAL EVALUATION

Sudhakar Patil* and S.S. Bhale

Organic Chemistry Laboratory, M.U. Mahavidyalay, Udgir- 413517
Email Id: ssbhale22@gmail.com

Abstract:
2, 4-Dioxo-4-thiophen-2-yl-butyric acid methyl ester**(2)**was synthesized from 2- acetyl thiophene by the treatment with diethyl oxalate. The 2, 4-Dioxo-4-thiophen-2-yl-butyric acid methyl ester was further converted to respective substituted pyrazole**(3-5)** by treatment with hydrazine hydrate, Semicarbahydrazie and Phenyl hydrazine. Similarly, 2, 4-Dioxo-4-thiophen-2-yl-butyric acid methyl ester treated with hydroxyl hydrochloride, followed by treatment with conc. HCl to form respective Isooxazole**(6)**.The structures of the compounds reported in the thesis have been confirmed by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and Mass spectra.Selected compounds have also been evaluated for their biological activities.

Keywords: 2-acetyl Thiophene, Isoxazole and Pyrazoles.

Introduction

2-acetyl Thiophene derivatives have been found to be veryreactive towards organic reagents such as hydroxyl aminehydrochloride, semicarbazide hydrochloride, hydrazinehydrate and phenyl hydrazine, hence they are utilized for thesynthesis of substituted isoxazoles and pyrazole carboxylates.

Compounds having a pyrazole nucleus are known to possesssome important pharmacological activities such as antitumor[I–IV], antibacterial[V], fungicidal[VI-VII], antidiuretic[VIII], anticancer[IX], potent antidiabeticagent[X], anti-inflammatory[XI], antidepressant[XII-XIII]andantiviral[XIV] activities. Some substituted pyrazoles are cycloxygenes-2-(Cox2) selective inhibitors[XV].

A literature survey indicatedthat pyrazole carboxylates when reacted with hydrazinehydrate yield pyrazole carbohydrazides[XVI-XVII]possessing interestingbioactivities such as antifungal[XVIII-XIX],antimalarial[XX], anticonvulsant[XXI], antituberculosis[XXII-XXIII]and anticancer[XXIV].

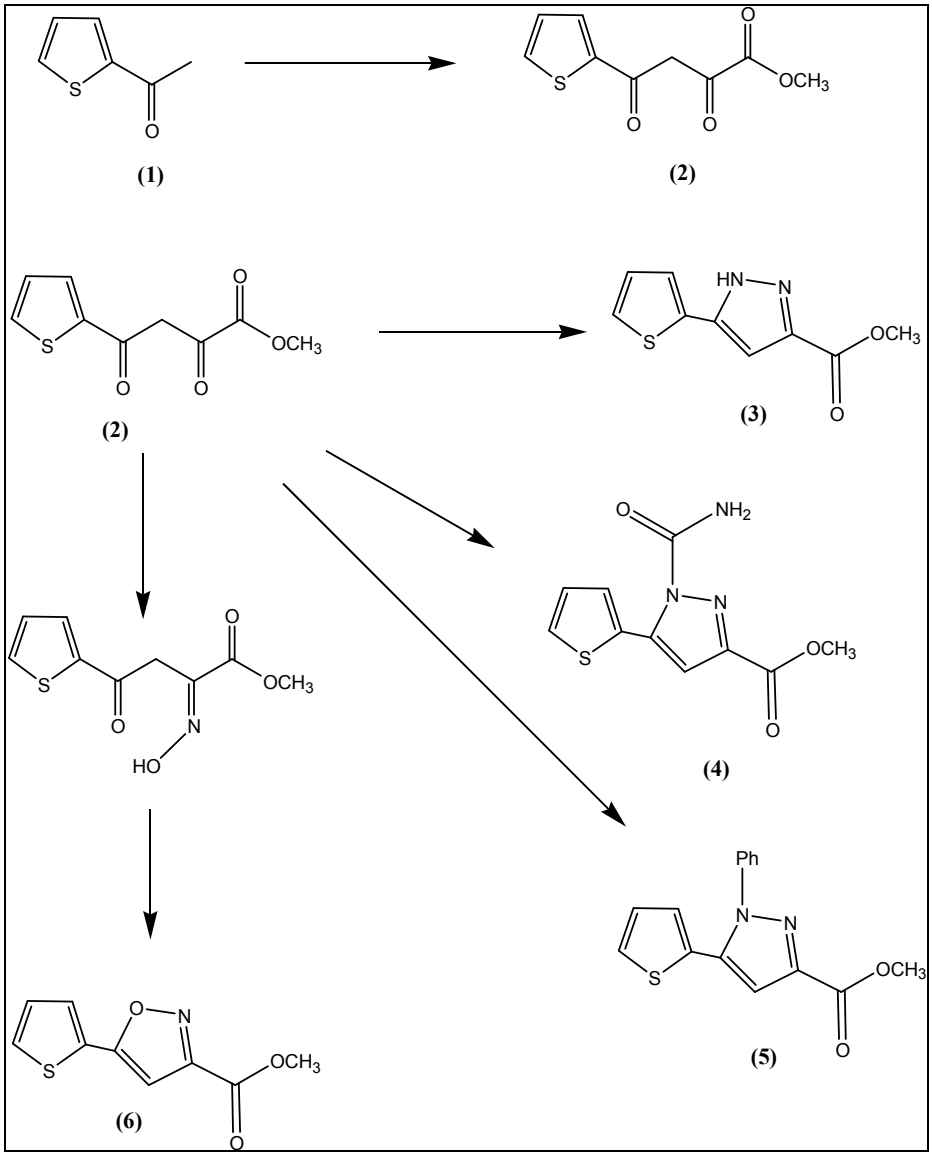
Pyrazole carbohydrazide reacts with different reagents to give1,3,4-oxadiazoles which have a broad spectrum of biologicaland industrial activities[XXV-XXVI]. Among the biological applications reported for 1,3,4-oxadiazoles are hypnotic[XXVII], anticancer[XXVIII], antituberculostatic[XXIX],antimalarial[XXX],antimicrobial[XXXI-XXXII], antiviral[XXXIII,XXXIV], hypoglycaemic[XXXV], anti-HIV activity[XXXVI], insecticidal[XXXVII], and antifungal[XXXVIII]activities.

In view of these reports and in continuation ofour previous work[XXXIX] we describe here a facile synthesis ofisoxazole and pyrazole-3-carboxylates from 2-acetyl thiophene**(1)**.

Result and Discussion:

The 2, 4-Dioxo-4-thiophen-2-yl-butyric acid methyl ester**(2)** was further converted to respective substituted pyrazole**(3-5)** by treatment with hydrazine hydrate, Semicarbahydrazie and Phenyl hydrazine. Similarly, 2, 4-Dioxo-4-thiophen-2-yl-butyric acid methyl ester treated with hydroxyl hydrochloride, followed by treatment with conc. HCl to form respective Isooxazole**(6)**. The structures of the compounds was established on the basis of spectral techniques also their antimicrobial activity was evaluated against gram positive as well as gram negative bacteria's.

Scheme I:



5-Thiophen-2-yl-1H-pyrazole-3-carboxylic acid methyl ester

Yield: 84 %, 220-23°C; IR (KBr) cm^{-1}: 1756 (C=O), ^{1}H NMR (DMSO-d_6, δ , ppm): 3.73 (s, 3H, OCH_3), 7.88-8.21 (m, 4H, Ar-H, CH); 9.59 (s, 1H, NH), ^{13}C NMR (DMSO-d_6, δ , ppm): 56.3 (OCH_3), 120.6-130.8 (C=C & Ar-C), 159.81 (C=N), 179.89 (C=O). Anal.% $C_9H_8N_2O_2S$: C, 51.91; H, 3.87; N, 13.45. Found: C, 51.14; H, 3.61; N, 12.93.

1-Carbamoyl-5-thiophen-2-yl-1H-pyrazole-3-carboxylic acid methyl ester
Yield: 65 %, 216-18°C; IR (KBr) cm^{-1}: 3310 (OH); 2201 (C=N), ^{1}H NMR (DMSO-d_6, δ, ppm): 3.87 (s, 3H, OCH_3), 6.15 (s, 2H, NH_2), 7.65-8.04 (m, 4H, Ar-H, CH); ^{13}C NMR (DMSO-d_6, δ, ppm): 53.78 (OCH_3), 122.41-131.21 (C=C & Ar-C), 159.21 (C=N), 175.89 (C=O), 176.15 (C=O). Anal.% $C_{10}H_9N_3O_3S$: C, 47.80; H, 3.61; N, 16.72. Found: C, 47.74; H, 3.57; N, 16.63.

1-Phenyl-5-thiophen-2-yl-1H-pyrazole-3-carboxylic acid methyl ester
Yield: 76 %, 203-05°C; IR (KBr) cm^{-1}: 1815 (C=O), ^{1}H NMR (DMSO-d_6, δ, ppm): 3.68 (s, 3H, OCH_3), 6.94-8.42 (m, 9H, Ar-H and CH); ^{13}C NMR (DMSO-d_6, δ, ppm): 56.21 (OCH_3), 124.12-133.24 (C=C & Ar-C), 167.9 (C=N), 175.64 (C=O). Anal.% $C_{15}H_{12}N_2O_2S$: C, 63.36; H, 4.25; N, 9.85. Found: C, 63.18; H, 4.11; N, 9.57.

5-Thiophen-2-yl-isoxazole-3-carboxylic acid methyl ester
Yield: 67 %, 212-14°C; IR (KBr) cm^{-1}: 1820 (C=O), ^{1}H NMR (DMSO-d_6, δ ppm): 3.87 (s, 3H, OCH_3), 7.42-8.32 (m, 4H, Ar-H, CH); ^{13}C NMR (DMSO-d_6, δ, ppm): 55.73 (OCH_3), 124.48-133.14 (C=C & Ar-C), 158.98 (C=N), 176.30 (C=O). Anal.% $C_9H_7NO_3S$: C, 51.67; H, 3.37; N, 6.69. Found: C, 51.48; H, 3.23; N, 6.56.

Experimental
Melting points were determined on a capillary melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in the indicated solvent on Joul 300 MHz spectrophotometer using TMS as an internal standard. Infrared spectra were recorded in Bruker FTIR spectrophotometer. Microanalyses were performed on Carlo Ebra 1108 element analyzer and were within the ± 0.5% of the theoretical values. Column chromatography was performed on silica gel (Merck, 100-200 mesh).

Synthesis of 2,4-Dioxo-4-thiophen-2-yl-butyric acid methyl ester (2)
Diethyloxalate (10 mmol) was gradually added withstirring to a solution of 2-acetyl Thiophene (10 mmol) andsodium methoxide (0.23 g Na in 5 mL methanol, 10 mmol) inN,N – Dimethylformamide (100 mL). The reaction mixture wasstirred for 12 hrs at room temp., the product obtained wasacidified by 1:1 ice-cold HCl, filtered, washed with water andrecrystallized from acetone to get yellow crystalline solid **2**
Yield = 85 %; m.p.: 131–133 °C;

Synthesis of 5-Thiophen-2-yl-1H-pyrazole-3-carboxylic acid methyl ester (3)
Hydrazine hydrate (30 mmol) was added graduallywith constant stirring to **2** (10 mmol) in CH_3COOH (30 mL), andrefluxed for 2 hrs. After that it was poured in ice-cold water, filteredand recrystallized from ethanol to get white crystalline solid**(3)**.

Synthesis of 1-Carbamoyl-5-thiophen-2-yl-1H-pyrazole-3-carboxylic acid methyl ester (4)
Semicarbazide hydrochloride (10 mmol) and sodium acetate (10 mmol) were added to **2** (5 mmol) in absoluteethanol (99.9 %, 10 mL), and the reaction mixture was refluxedfor 4 h. It was then concentrated, cooled and poured in ice-coldwater, solid separated out was filtered and recrystallized fromethanol to get white crystalline solid**(4)**.

Synthesis of 1-Phenyl-5-thiophen-2-yl-1H-pyrazole-3-carboxylic acid methyl ester (5)
Phenyl hydrazine (15 mmol) was added to a mixtureof **2** (10 mmol) in CH_3COOH (30 mL) and the reaction mixture wasrefluxed for 4 hrs. After that it was concentrated and poured incrushed ice, filtered off and recrystallized from acetic acid aswhite crystalline solid**(5)**.

5-Thiophen-2-yl-isoxazole-3-carboxylic acid methyl ester (6)
Hydroxylamine hydrochloride (20 mmol) and sodiumacetate (20 mmol) were added to a mixture of **2** (10 mmol) inabsolute ethanol (99.9 %, 200 mL) and the reaction mixture wasrefluxed for 4 hrs. It was concentrated, cooled, poured in ice-coldwater and kept overnight; the solid separated out was filteredand recrystallized from diluted ethanol to get 6a, as an intermediate.Further, 6a was refluxed for 2 hrs inabsolute ethanol (50 mL)and conc. HCl (1 mL). The solvent was evaporated underreduced pressure to get pale yellow crystalline solid **(6)**recrystallized from ethanol.

Antimicrobial and antifungal activities
All the newly synthesized compounds were evaluated for their antibacterial activity against gram-negative bacteria, E coli and P aeruginosa and gram-positive bacteria, S aureus, and C diphtheriae using disc diffusion method. The zone of inhibition was measured in mm and the activity was compared with standard drug. The data is given in following **Table 1**.

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Table I: Antimicrobial activity of Synthesized compounds

Compds	**Zone of inhibition (in mm)***			
	Gram Positive		**Gram Negative**	
	S.aureus	***C. diphtheria***	***E. coli***	***P. aeruginosa***
2	17	18	17	17
3	24	23	18	16
4	21	20	20	20
5	18	18	15	17
6	23	20	17	15
Ciprofloxacin	25	24	24	22
DMSO	0	0	0	0

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