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RESEARCH ARTICLE

Synthesis and Antimicrobial Activity of Some Isoxazole Derivatives of Thiophene

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Abstract: A series of new chalcones were synthesized by the reaction of 2-acetyl thiophene and substituted bezaldehydes. Then the chalcones were made to undergo cyclization reaction with hydroxylamine hydrochloride in ethanol to afford the synthesis of isoxazole derivatives of thiophene. The synthesized compounds were purified and their structures were elucidated with the help of IR and ^{1}H NMR spectroscopy. The compounds were screened for antimicrobial activity by disc diffusion method. The result suggested that the four compounds were moderately to highly active.

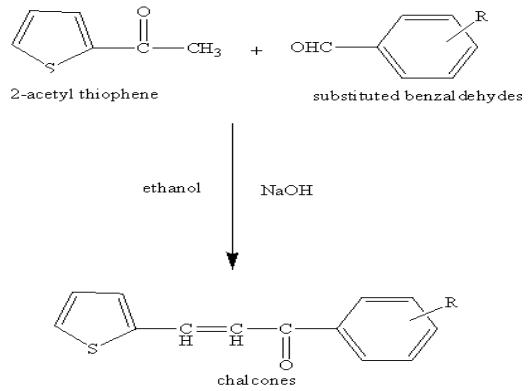
Keywords: 2-Acetyl thiophene, Chalcones, Hydroxylamine hydrochloride, Isoxazoles, Antimicrobial activity

Introduction

Isoxazoles have been reported to possess diverse biological activities like anti-inflammatory[1], antibacterial[2], antifungal[3], antibiotic[4], anticonvulsant[5], antitubercular[6], anxeolytic[7] properties. Ngaini *et al.*[8] reported the various activities of chalcones, exhibiting anti-inflammatory, antimicrobial, anti malarial, anticancer, antioxidant, antihyperglycemic, antiangiogenic and antiplatelet activities. A little regarding the introduction of thiophene moiety to isoxazole ring has been reported, therefore, it was thought worthwhile to synthesis some new isoxazole derivatives attached to thiophene heterocyclic ring and evaluate them for their antimicrobial activity.

Experimental

The chemical synthesis was initiated by synthesizing different chalcones (Scheme 1) by the literature method[9]. A summary of synthesized chalcones are described in the Table 1. Then the isoxazole derivatives were prepared by adopting a general method in which the synthesized chalcones were treated with hydroxylamine hydrochloride in ethanol to afford the synthesis of isoxazole derivatives of thiophene (Scheme 2).



R= 4-C_8H_7O, 3-NO_2 4-NO_2, 3-OCH_3, 3-Cl, 3-CH_3

Scheme 1. Synthesis of chalcones

Table 1. General structure of synthesized chalcones

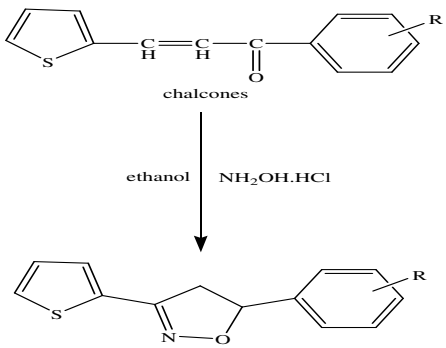
S. No.	Intermediate Compound Code	R	Molecular Formula	M.P., ^oC	Yield, %	R_f Value	Solvent system
1.	P-1	4-C_8H_7O	$C_{20}H_{16}O_2S$	59-60	60%	0.73	*n*-hexane:ethyl acetate, 2:1
2.	P-2	4-NO_2	$C_{13}H_9NO_3S$	134-135	55%	0.60	*n*-hexane:ethyl acetate, 2:1
3.	P-3	3-OCH_3	$C_{14}H_{12}O_2S$	61-62	70%	0.79	*n*-hexane:ethyl acetate, 2:1
4.	P-4	3-NO2	$C_{13}H_9O_3S$	129-130	60%	0.65	*n*-hexane:ethyl acetate, 2:1
5.	P-5	3-Cl	$C_{13}H_9ClOS$	79-80	65%	0.84	*n*-hexane:ethyl acetate, 2:1
6.	P-6	3-CH_3	$C_{14}H_{12}OS$	69-70	55%	0.88	*n*-hexane:ethyl acetate, 2:1

To a solution of different (P_1-P_6) chalcones (0.01 mole) in ethanol and anhydrous sodium acetate (0.81 g, 0.01 mole) dissolved in minimum amount of acetic acid, solution of hydroxylamine hydrochloride was added. The reaction mixture was refluxed on oil bath for hours. The completion of reaction was monitored by TLC. After the completion of reaction, the solution was cooled to get the products, which were purified by recrystallization from ethanol. The synthesized thiophene derivatives are described in Table 2.

Table 2. Physical parameters of synthesized compounds

S. No.	Compd Code	R	Molecular Formula	M.P., ^oC range	Yield, %	R_f value	Reaction time, h
1.	KCG-1	4-C_8H_7O	$C_{20}H_{17}NO_2S$	71-72	60%	0.58	15
2.	KCG-2	4-NO_2	$C_{13}H_{10}N_2O_3S$	134-135	70%	0.72	10
3.	KCG-3	3-OCH_3	$C_{14}H_{13}NO_2S$	< 40	50%	0.62	12
4.	KCG-4	3-NO_2	$C_{13}H_{10}N_2O_3S$	< 40	55%	0.70	10
5.	KCG-5	3-Cl	$C_{13}H_{10}ClNOS$	< 40	65%	0.84	8
6.	KCG-6	3-CH_3	$C_{14}H_{13}NOS$	< 40	55%	0.91	10

Mobile phase: n-hexane: ethyl acetate: 2:1



4,5-dihydro-5(substituted phenyl)-(3-thiophene-2yl)isoxazoles

R= 4-C_8H_7O, 3-NO_2 4-NO_2, 3-OCH_3, 3-Cl, 3-CH_3

Scheme 2. Synthesis of isoxazoles

Spectral data of synthesized compounds

4, 5-Dihydro-5-(4-benzyloxyphenyl)-3-(thiophene-2-yl)isoxazole (KCG-1)

IR (KBr cm^{-1}):2909(-CH_2-), 1509(=C=N-O), 1225(C-O) and712(C-S-C). ^{1}H NMR ($CDCl_3$): δ1.2009(s, 2H, isoxazole ring), δ 4.985-5.090(t, 2H, benzyl) and δ 6.918-8.018(m, 12H, Ar-H)

4, 5-Dihydro-5-(4-nitrophenyl)-3-(thiophene-2-yl)isoxazole (KCG-2)

IR (KBr cm^{-1}):2937(-CH_2-), 1510(=C=N-O), 1344(NO_2), and 714(C-S-C). ^{1}H NMR ($CDCl_3$): δ1.214(s, 2H, isoxazole ring) and δ 8.694-8.001 (m, 7H, Ar-H)

4, 5-Dihydro-5-(3-methoxyphenyl)-3-(thiophene-2-yl)isoxazole (KCG-3)

IR (KBr cm^{-1}):2960(-CH_2-), 1597(=C=N-O), 1256(C-O) and 775(C-S-C). ^{1}H NMR ($CDCl_3$): δ1.225 (s, 2H, isoxazole ring), 3.633-3.842(t, 3H, OCH_3) and δ 7.081-7.7.859 (m, 7H, Ar-H)

4, 5-Dihydro-5-(3-nitrophenyl)-3-(thiophene-2-yl)isoxazole (KCG-4)

IR (KBr cm^{-1}):2917(-CH_2-), 1605(=C=N-O), 1350(NO_2), and772(C-S-C). ^{1}H NMR ($CDCl_3$): δ1.240(s, 2H, isoxazole ring) and δ 8.095-8.655 (m, 7H, Ar-H)

4, 5-Dihydro-5-(3-chlorophenyl)-3-(thiophene-2-yl)isoxazole (KCG-5)

IR (KBr cm^{-1}):2920(-CH_2-), 1598(=C=N-O), 1350(C-Cl aryl) and 772(C-S-C). ^{1}H NMR ($CDCl_3$):δ1.225(s, 2H, isoxazole ring) and δ 7.011-7.8860 (m, 7H, Ar-H)

4, 5-Dihydro-5-(3-methylphenyl)-3-(thiophene-2-yl)isoxazole (KCG-6)

IR (KBr cm^{-1}):3015(-CH_3), 2917(-CH_2-), 1581(=C=N-O) and 773(C-S-C). ^{1}H NMR ($CDCl_3$): δ1.260 (s, 2H, isoxazole ring), δ 4.120(s, 3H, CH_3) and δ 7.015-7.868 (m, 7H, Ar-H)

Antibacterial activity

The antibacterial activity was assayed by agar plate disc diffusion method[10] at the concentration of 50 µg per disk. All the synthesized compounds were tested *in vitro* for their antibacterial activity against gram positive microorganisms such as *Staphylococcus aureus, Bacillus subtilis* and gram negative *Escherichia coli, Pseudomonas aerugenosa* strains. Each test compounds were dissolved in dimethyl sulphoxide (DMSO) to get required concentration. The discs (6 mm in diameter) were impregnated , air dried and placed on the agar medium, previously seeded with 0.2 mL of broth culture of each organism for 18 h.

The plates were incubated at 37 ^{o}C for 24 h and the inhibition zones were measured in mm. Discs impregnated with DMSO were used as a control and ciprofloxacin discs as antibacterial reference standard.

Antifungal activity

The antifungal activity[11] was assayed by sabouraud dextrose agar media plate disc diffusion method at the concentration of 50 µg per disk. All the synthesized compounds were tested *in vitro* for their antifungal activity against microorganisms such as *Asperagellus niger* and *Candida albicans*. Each test compound was dissolved in dimethyl sulphoxide (DMSO) to get required concentration. The discs (6 mm in diameter) were impregnated; air dried and placed on the sabouraud dextrose agar media, previously seeded with 0.2 mL of broth culture of each organism for 18 h. The plates were incubated at 22 ^{0}C for 48 h and the inhibition zones were measured in mm. Discs impregnated with DMSO were used as a control and fluconazole discs as antifungal reference standard. The result of activity was described in the Table 3.

Table 3. Antifungal activity of the compounds

Compound 50 µg/disc	Zone of inhibition in mm					
	B.subtilis	*S.aureus*	*E. coli*	*P.aeruginosa*	*A. niger*	*C. albicans*
KCG-1	++	++	++	++	++	++
KCG-2	-	-	-	-	-	-
KCG-3	-	++	-	+	-	-
KCG-4	++	++	++	++	++	+
KCG-5	++	++	+++	+++	++	+++
KCG-6	++	++	++	++	++	+++
Ciprofloxacin	+++	+++	+++	+++		
Fluconazole					+++	+++
Solvent control DMSO	-	-	-	-	-	-

Inactive (inhibition zone < 6 mm); slightly active = '+' (inhibition zone 7-9 mm); moderately. Active = '++' (inhibition zone 10-13 mm); highly active = '+++' (inhibition zone > 14 mm)

Results and Discussion

The IR spectra of the synthesized compounds (KCG 1-KCG 6) clearly showed the formation of isoxazoline ring by the appearance of band at 1509-1600 cm^{-1} (=C=N-O) stretching vibration and the attachment of Thiophene ring was confirmed by the appearance of band at 714-775 cm^{-1} (C-S-C) stretching vibration. The ^{1}H NMR data also confirmed the synthesis by showing δ values for various hydrogen. The ^{1}HNMR spectra showed a singlet at about δ 1.225 for 2H, isoxazole ring and multiplets for the aromatic hydrogen at about δ 7.0-7.8. The attachment of various substituent groups in the synthesized compounds were also validated by their respective IR and ^{1}H NMR spectra. The compounds KCG-5 was found to be highly active against *E. coli, P. aeruginosa* and *C. albicans.* The enhanced activity of this compound may be attributed to the presence of chloride as a substituent on the aromatic ring collectively increasing the lipid solubility. The rest of the compounds KCG-1, KCG-4 and KCG-6 were found moderately to highly active.

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