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Synthesis and Biological activity of some Novel Chalcone derivatives containing [1, 3, 4] oxadiazole-2(3H)-thione

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Abstract

Some new and biologically active chalcone derivatives were synthesized by Claisen Schmidt condensation of different benzaldehyde derivatives, synthesized from 5-p/o/m-tolyl-1, 3, 4-oxadiazole-2(3H)-thione with acetophenone. The structure of the synthesized compounds has been established on the basis of IR, ^{1}HNMR and elemental analysis. The compounds have been evaluated for antibacterial activity against Bacillus subtilis, Staphylococcus aureus, Pseudomonas aureginosa and Escherichia coli and antifungal activity against Aspergillus flavus, Aspergillus niger, Fusarium oxysporum and Collatotrichum falcatum.

Keywords: Chalcone, oxadiazolethione, antibacterial activity and antifungal activity.

Introduction

The Chalcones are α, β-Unsaturated ketones, containing the reactive ketoethylenic group –CO–CH=CH–, especially 1, 3-diarylprop-2-en-1-ones, has relatively low redox potentials and have a greater probability of undergoing electron transfer reactions due to a conjugated double bonds and an entirely delocalized π-electron system on both benzene rings, which is responsible for their biological activity and that's why it have received considerable attention in medicinal chemistry[1]. Chalcones, either natural or synthetic, are known to exhibit various biological activities such as antimicrobial[2-7], anti-inflammatory[8-11], analgesic[12], antiplatelet[13], antiulcerative[14], antimalarial[15-19], anticancer[20-26], antitumor[27-29], antiviral[30], antileishmanial[31,32], antioxidant[33,34], antitubercular[35-37], antihyperglycemic[38], immunomodulatory[39], antibacterial[40,41], antiplasmodial[42], antifungal[43-45] activities.

Chalcones constitute an important group of natural products and some of them possess a wide range of biological activities. The diverse properties of chalcones have prompted us to synthesize novel chalcone derivatives in order to study their biological activity. The desired compounds 4a-f have been synthesized in 38-46% yield, by selective oxidation of methyl group of compound 3 into –CHO, with chromyl chloride and followed by Claisen Schmidt condensation with acetophenone furnished the compound 4 (Scheme-1). The characteristic and spectral data of different title compound 4 synthesized are recorded in table-1.

Material and Methods

Melting points were determined in open capillaries and are uncorrected. IR spectra in KBr were recorded on a Perkin-Elemer 881 infrared spectrophotometer (λ_{max} cm^{-1}) and ^{1}H NMR spectra in DMSO-d6 on a Bruker Avance DPX 200 MHz spectrometer using TMS as internal reference. All the starting materials were synthesized by the method given in literature and identified by ^{1}H NMR spectra, IR spectra and micro analysis of these compounds was in satisfactory agreement with the structures. The purity of the compounds was monitored by TLC using pre-coated silica gel plate.

General Procedure: Synthesis of substituted benzoic acid hydrazides 2a-f: The acid hydrazides have been prepared by the conventional method. 4-methyl benzoic acid 1a (10 g, 0.07 mole) was dissolved in 120 ml of n- butanol and slowly added 8 ml of conc. sulfuric acid. The reaction mixture was refluxed for 6-7 hrs by using Dean-Stark apparatus. After cooling, the reaction mass was treated with saturated solution of sodium carbonate. The organic layer was separated out, washed with water and concentrate up to 20-30 mL by applying vacuum distillation. A mixture of concentrated reaction mass and hydrazine hydrate (10 ml, 0.2 mole) in 70 ml methanol was refluxed on water bath for 9 hrs. After cooling, the reaction mass was poured in to water. The desired product thus obtained was filtered, washed with water and purified by the recrystallization from ethanol.

Synthesis of 5-Aryl-1, 3, 4-oxadiazole-2(3H)thione 3a-f: A mixture of **2a** (1.0g, 0.006 mole) and carbon disulphide (18mL) and pyridine (20mL) was heated under reflux on water bath for 9-10 hrs. After cooling, the solvent was evaporated under reduced pressure and the residue was triturated with an ice-water mixture and neutralized with diluted HCl. The desired product thus obtained was filtered, washed with water and purified by the recrystallization from ethanol to afford 3a (Yield=70%, M.P.=242-245^{0}C); 3b (Yield=76 %, M.P.=135-140^{0}C); 3c (Yield=41%, M.P.=185-190^{0}C); 3d (Yield= 93 %, M.P.=204-208^{0}C); 3e (Yield=68%, M.P.=253-257^{0}C), 3f (Yield=65%, M.P.=215-218^{0}C).

Synthesis of 1-phenyl-3-(4-(5-thioxo-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl) phenyl) prop-2-en-1-one (4a-d):
Synthesis of 1-phenyl-3-(3-(5-thioxo-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl) phenyl) prop-2-en-1-one (4e):
Synthesis of 1-phenyl-3-(2-(5-thioxo-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl) phenyl) prop-2-en-1-one (4f):

Compound of 3a (0.6g, 0.003mole) was dissolved in carbon tetra chloride (10mL) and added freshly prepared chromyl chloride (2mL dissolved in carbon tetra chloride of 10mL) drop wise under stirring at 0-5^{o}C. The reaction mixture was stirred at 0-5^{o}C for 2 hrs and remove the external cooling and allow the reaction mass stirred at 25-30^{0}C for 1-2 hrs. Finally, the reaction mass was heated under reflux on water bath for 11hrs. The reaction completion was monitored by Fehling test of –CHO group because the product (having –CHO group) spot is not visible on TLC plate and hence formation of product is confirmed by Fehling test of –CHO group and consumption of starting material by TLC. After cooling, the excess carbon tetrachloride was evaporated from reaction mass and quenched with chilled saturated solution of sodium sulphite. The obtained precipitate of basic chromium salt was treated with 1:1 dil HCl and extracted by dichloromethane. The organic layer was distilled off to get crude. A mixture of obtained crude and acetophenone (0.36mL, 0.003mol) was dissolved in ethanol (20mL), added sodium ethoxide solution (prepared by 0.1 gm Na in10 mL dry ethanol) drop wise under stirring at 0-5^{0}C and allow stirring for 8-9 hrs at 30-35^{0}C. The reaction mass was poured into ice water and neutralized with dil HCl. The desired product thus obtained was filtered, washed with water and purified by the recrystallization from ethanol to afford 4a (Yield =42%, M.P. = 175^{0}C).

Spectral data of Compund 4: 1-phenyl-3-(4-(5-thioxo-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl)phenyl)prop-2-en-1-one 4a: IR (KBr, cm^{-1}): 1650(>C=O ketone), 1262(>C=S), 1620(>C=N), 3256(-NH), 1615(CH=CH ethylene), 1592(CH=CH aromatic), 1176(C-O-C).
^{1}H NMR (DMSO-d_6) (δ, ppm): 7.40-7.82 (m, 9H aromatic C-H), 7.31(d, 1H, –COCH=, ethylene proton) 7.90(d, 1H, -PhCH=, ethylene proton), 10.40(s, 1H, NH).

1-phenyl-3-(2-nitro-4-(5-thioxo-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl)phenyl)prop-2-en-1-one 4b: IR(KBr,cm^{-1}): 1657(>C=O ketone),1265(>C=S), 1620(>C=N) 3250(-NH), 1615(CH=CH ethylene), 1600(CH=CH aromatic), 1176(C-O-C).
^{1}H NMR (DMSO-d_6) (δ, ppm): 7.61- 7.90(m, 8H aromatic C-H), 7.59(d, 1H –COCH=, ethylene proton), 8.60(d, 1H PhCH=, ethylene proton), 10.30(s, 1H, NH).

1-phenyl-3-(2, 6-dinitro-4-(5-thioxo-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl)phenyl)prop-2-en-1-one 4c: IR(KBr, cm^{-1}): 1650(>C=O ketone), 1258(>C=S), 1620(>C=N) 3266(-NH), 1610(CH=CH ethylene), 1590(CH=CH aromatic), 1170(C-O-C).
^{1}H NMR (DMSO-d_6) (δ, ppm):7.61- 8.42(m, 7H aromatic C-H), 7.61(d,1H –COCH=,ethylene proton), 8.50(d,1H PhCH=,ethylene proton), 10.39 (s,1H,NH).

1-phenyl-3-(2-bromo-4-(5-thioxo-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl)phenyl)prop-2-en-1-one 4d: IR(KBr, cm^{-1}): 1650(>C=O ketone), 1260(>C=S), 1618(>C=N) 3260(-NH), 1615(CH=CH ethylene), 1600(CH=CH aromatic), 1173(C-O-C).
^{1}H NMR (DMSO-d_6) (δ, ppm): 7.41-7.82(m, 8H aromatic C-H), 7.36(d, 1H –COCH=, ethylene proton), 8.19(d, 1H PhCH=, ethylene proton), 10.35 (s, 1H, NH)

1-phenyl-3-(3-(5-thioxo-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl)phenyl)prop-2-en-1-one 4e: IR(KBr, cm^{-1}): 1648(>C=O ketone), 1262(>C=S), 1618(>C=N), 3256(-NH), 1615(CH=CH ethylene), 1590(CH=CH aromatic), 1176(C-O-C).
^{1}H NMR (DMSO-d_6) (δ, ppm): 7.42-7.82(m, 9H aromatic C-H), 7.51(d, 1H –COCH=, ethylene proton), 8.02(d, 1H PhCH=, ethylene proton), 10.40 (s, 1H, NH)

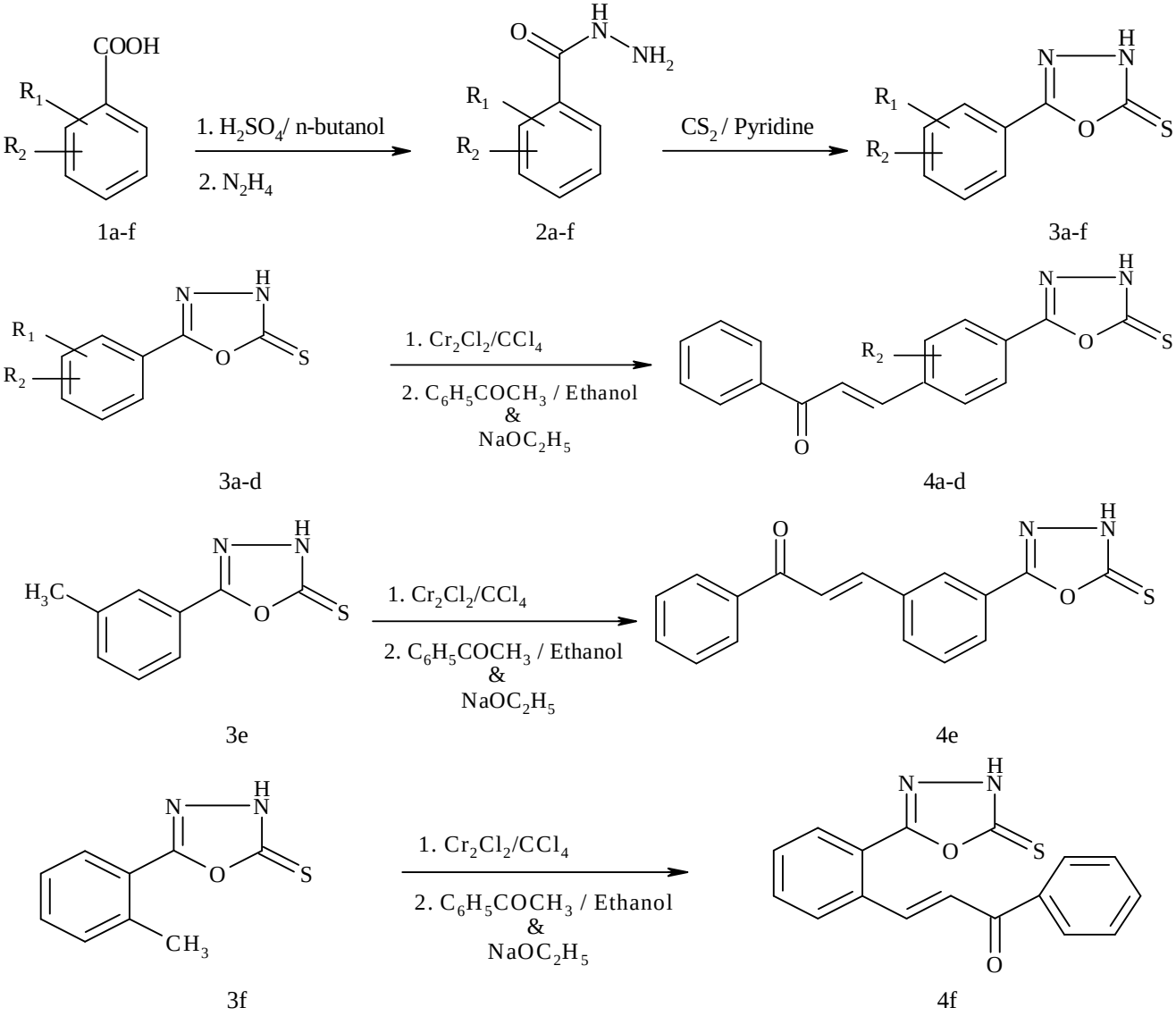
1-phenyl-3-(2-(5-thioxo-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl)phenyl)prop-2-en-1-one 4f: IR(KBr, cm^{-1}): 1650(>C=O ketone), 1262(>C=S), 1620(>C=N), 3290(-NH), 1610(CH=CH ethylene), 1600(CH=CH aromatic), 1176(C-O-C).
^{1}H NMR (DMSO-d_6) (δ, ppm): 7.40- 7.82(m 9H aromatic C-H) 7.24(d, 1H –COCH=, ethylene proton), 7.83(d, 1H PhCH=, ethylene proton), 10.40 (s, 1H, NH).

Evaluation of antimicrobial activity: The synthesized compounds were tested for their in vitro antibacterial activity against the Gram-positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* bacteria. The antifungal activity was evaluated against *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum* and *Colletotrichum falcatum*. The MIC values were determined by using disc diffusion technique in NAM (nutrient agar medium) for the antibacterial activity and Czapex agar medium for antifungal activity. All compounds were tested at concentration 10mg/mL, 5mg/mL, 2.5mg/mL, 1.25mg/mL, 0.62mg/mL, 0.31mg/mL and 0.15mg/mL. Ampicillin was used as the reference antibacterial agents, whereas Fluconazole was used as the reference antifungal agents. The standard solution of different concentration of each compound was prepared by dissolving 20 mg compound into 2mL DMSO (Dimethyl sulfoxide) (10mg/mL). 1mL of this solution is diluted with 1mL DMSO to get 5mg/mL and similarly dilution was repeated to get 2.5 mg/mL, 1.25 mg/mL, 0.62 mg/mL, 0.31 mg/mL and 0.15mg/mL respectively. A reference standard for both gram positive and gram negative bacteria was made by dissolving accurately weighed quantity of Ampicilline and for fungi was made by dissolving accurately weighed quantity of Fluconazole in DMSO to get 10mg/mL, 5mg/mL, 2.5mg/mL, 1.25mg/mL, 0.62mg/mL, 0.31mg/mL and 0.15mg/mL respectively. The incubation was carried out at 37ºC for 24h for bacterial growth and at 37ºC for 48h for fungi. All the experiments were carried

out in triplicate. Simultaneously, controls were maintained by employing 0.1 mL of dimethyl sulfoxide which did not reveal any inhibition. The results of antibacterial and antifungal studies are given in table-2.

Result and Discussion

The antimicrobial data showed that all the synthesized compounds are fairly active. The antibacterial results revealed that some of the synthesized compounds showed varying degrees of inhibition against Gram positive bacteria shown in table-2. The 4c and 4e showed excellent activity against *Staphylococcus aureus* at concentration 0.15mg/mL whereas 4a, 4b, 4d and 4f including reference standard ampicilline are active at concentration 0.31mg/mL. The compounds 4b and 4e are fairly active as reference standard ampicilin at concentration 0.15mg/mL against *Bacillus subtilis.* The compound 4a, 4d and 4f showed activity against *Bacillus subtilis* at 0.62mg/mL, whereas 4c at 0.31mg/mL. All compounds are found active at slightly higher concentration (0.31mg/mL-1.25mg.mL) against *Pseudomonas aureginosa* and *E. coli* as compare to standard ampicilline. All compounds showed activity against fungi *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum and Collatotrichum falcatum* at just higher concentration than standard fluconazole, where as 4c and 4e are found active at 0.31mg/mL against *Fusarium oxysporum.*



1a-f 2a-f 3a-f

3a-d 4a-d

3e 4e

3f 4f

a: R_1=4-CH_3; R_2=H
b: R_1=4-CH_3; R_2=3-NO_2
c: R_1=4-CH_3; R_2=3,4-diNO_2
d: R_1=4-CH_3; R_2=3-Br
e: R_1=3-CH_3; R_2=H
f: R_1=2-CH_3; R_2=H

Scheme-1

Table-1
Characterization data of compounds 4a-f

Compound	Yield (%)	m.p. ^{0}C	R_f*	Mol. Formula	Found (Calcd)		
					C	H	N
4a R_2= H	42	175	0.79	$C_{17}H_{12}N_2O_2S$	66.22 (66.18)	3.92 (3.90)	9.08 (9.05)
4b R_2= 3-NO_2	44	162	0.72	$C_{17}H_{11}N_3O_4S$	57.78 (57.75)	3.12 (3.09)	11.64 (11.60)
4c R_2= 3,5-diNO_2	46	160	0.65	$C_{17}H_{10}N_4O_6S$	51.06 (51.05)	2.43 (2.41)	14.06 (14.02)
4d R_2= 3-Br	38	168	0.63	$C_{17}H_{11}BrN_2O_2S$	52.53 (52.55)	2.85 (2.81)	7.22 (7.19)
4e	41	170	0.62	$C_{17}H_{12}N_2O_2S$	66.21 (66.18)	3.89 (3.90)	9.08 (9.05)
4f	43	181	0.67	$C_{17}H_{12}N_2O_2S$	66.23 (66.18)	3.91 (3.90)	9.03 (9.05)
Solvent system: Ethyl acetate-pet. Ether (1 : 5)							

Table-2
MIC (mg/mL) value of compounds 4a-f

Compound	Antibacterial activity				Antifungal activity			
	Gram positive		Gram negative					
	Bacillus subtilis	Staphylococcus aureus	Pseudomonas aureginosa	Escherichia coli	Aspergillus flavus	Aspergillu niger	Fusarium oxysporum	Collatotrichum falcatum
4a	0.62	0.31	1.25	1.25	0.62	1.25	1.25	0.62
4b	0.15	0.31	0.62	0.62	1.25	2.5	0.62	0.62
4c	0.31	0.15	0.62	0.31	1.25	2.5	0.31	0.31
4d	0.62	0.31	0.31	0.62	2.5	2.5	0.62	0.31
4e	0.15	0.15	0.31	0.31	1.25	1.25	0.31	0.62
4f	0.62	0.31	1.25	0.62	2.5	1.25	0.62	0.62
Ampicillin	0.15	0.31	0.15	0.15	-	-	-	-
Fluconazole	-	-	-	-	0.62	0.31	0.31	0.15

Conclusion

The synthesized compounds were characterized and the results obtained are confirmed that the desired products had formed. The antifungal activities of synthesized compounds were determined against four fungal species *Aspergillus flavus, Aspergillus niger*, *Fusarium oxysporum* and *Colletotrichum falcatum.* The antibacterial activities of synthesized compound were determined against the Gram-positive *Staphylococcus aureus, Bacillus subtilis* and Gram-negative *Escherichia coli, Pseudomonas aeruginosa*. All synthesized compounds 4a-f are found fairly active against bacteria and fungi both and their experimental data have been reported in table-2.

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