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[c024]

Synthesis and anti-microbial screening of 3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-5-methylphenyl)prop-2-en-one and its heterocyclic analogs.

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Abstract: Chalcones and their heterocyclic analogues are known to possess a broad spectrum of biological effects. The present study is devoted to the synthesis of 3,4-Dimethoxy-2'-Hydroxy-5'-methyl chalcone and its derived products i.e. flavanone, flavonol, flavone, aurone, isoxazoline, chalconeimine and acetoxy chalcone. These newly synthesized compounds were screened for their antibacterial and anti-fungal activities.

Keywords: chalcone, heterocyclic analogs, anti-microbial activity screenings.

Introduction:

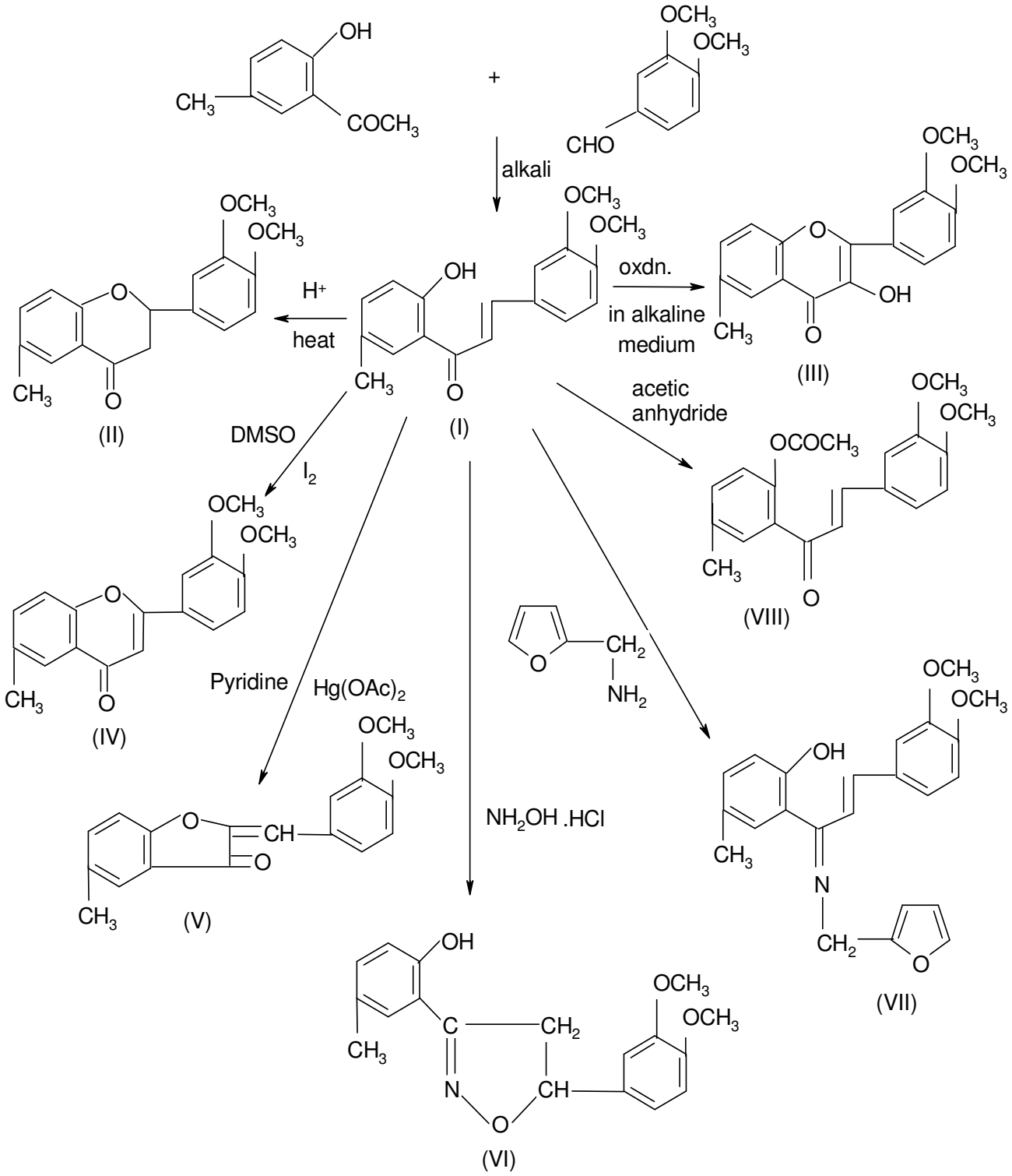
Flavonoids are an important group of naturally occurring bioactive compounds. This field of investigation was initiated in 1936 [1-2] by the discovery of *Citrin*, known as "Vitamin P or Permeabilitats Vitamin". It has since been claimed that many other flavonoids have similar pharmacological properties. Chalcones belong to an important class of flavonoids, which may be prepared by Claisen reaction. They possesses a wide range of biological activities and industrial applications. Kostanecki[3] was the first to give the term chalcone and who did pioneering work in the synthesis of naturally colouring compounds. The presence of enone function in the chalcone molecule confers antibiotic activity [4-6] (bacterio-static / bactericidal) upon it. Antibiotic activity is associated with the C=C bond of the chalcone molecule.

Chalcones and derivatives show a profound influence on the cardiovascular, cerebrovascular, and neurovascular systems, including the vital organs of the animals. Thus some chalcone have been associated with anti-peptic ulcer[7-9], hypertensive/antihypertensive [10] activity. Some other activities also have been reported

as anti-splasmodic and transquilizing action[11-12], anti-inflammatory[13], analgesic and sedative[14], anti-thrombic[12], capillary fragility[15-16], vasodilatory[17], estrogenic[18-19], anesthetic[20], anti-coagulating[21], anti-convulsant therapeutic[22], diuretic[23], anti-cancer[24] and anti-tubercular[25] activity. Some chalcone derivatives shows herbicidal activity[25] and substituted chalcones have been exhibited fungistatic and fungicide activity[26-28]. Since a broad spectrum of biological activities are associated with the above mentioned compounds, it was considered worthwhile to synthesize and evaluate anti-microbial activity of 3,4-Dimethoxy-2'-Hydroxy-5'-methyl chalcone and its derivatives.

Result and Discussion:

In the present work we have decided to carry out the synthesis of 3,4-Dimethoxy-2'-Hydroxy-5'-methyl chalcone (I) from 2-Hydroxy-5-methyl acetophenone and 3,4-Dimethoxy benzaldehye employing Claisen reaction. Flavanone (II) was obtained in acidic medium and flavonol (III) in alkaline medium from this chalcone. Chalcone undergoes cyclization in presence of heat to flavone (IV) and in presence of alkali furnishes aurone (V). Isoxazoline(VI) was obtained by the reaction of hydroxyl amine hydrochloride with the chalcone. Condensation of furfuryl amine with 3,4-Dimethoxy-2'-Hydroxy-5'-methyl chalcone resulted the corresponding chalconeimine (VII). Acetylation of the chalcone yields 2'- Acetoxy-3,4-dimethoxy-5'-methyl chalcone (VIII). The synthesis of these compounds was achieved following the linear pathway strategy summarized in **Scheme 1**. The MICs (Minimum inhibitory concentration) of these compounds in µg/ml was tested on bacteria namely namely *Escherichia coli (ATCC 9637)*, *Pseudomonas aeruginosa (ATCC BAA-427), Staphylococus aureus (ATCC 25923)* and *Klebsiella pneumoniae (ATCC 27736)* in Muller Hinton Broth by NCCLS method and fungi namely *Candida albicans, Cryptococus neoformans, Sporothrix schenckii, Trichopyton mentagrophytes, Aspergillus fumigatus* and *Candida parapsilosis (ATCC 22019)* by NCCLS method in RPMI 1640 medium All the compounds have shown MIC either =50 µg/ml or >50 µg/ml as shown in **Table 1** and **Table 2**.


OH
CH3
COCH3
+
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OCH3
CHO
alkali
OCH3
OCH3
OH
CH3
O
(I)
H+
heat
(II)
oxdn.
in alkaline
medium
(III)
acetic
anhydride
OCOCH3
(VIII)
DMSO
I2
(IV)
Pyridine
Hg(OAc)2
(V)
NH2OH .HCl
(VI)
CH2
NH2
(VII)
N

Scheme 1

Table 1: Antibacterial activity screening of some of the synthesized compounds i.e MICs in µg/ml

S.No	Compound No	Minimum inhibitory conc. (MIC) in µg/ml			
		E. coli	*P. aeruginosa*	*S. aureus*	*K. pneumonia*
1	**I**	>50	>50	>50	>50
2	**II**	>50	>50	>50	>50
3	**III**	>50	>50	>50	>50
4	**IV**	>50	>50	>50	>50
5	**V**	>50	>50	>50	>50
6	**VI**	>50	>50	>50	>50
7	**VII**	>50	>50	>50	>50
8	**VIII**	>50	>50	>50	>50

Table 2: Antifungal activity screening of some of the synthesized compounds i.e MICs in µg/ml

S.No	Compound No	Minimum inhibitory conc. (MIC) in µg/ml					
		C. albicans	*C. neoformans*	*S. schenckii*	*T. mentagrophytes*	*A. fumigatus*	*C. parapsilosis*
1	**I**	>50	>50	>50	>50	>50	>50
2	**II**	>50	>50	>50	50	>50	>50
3	**III**	>50	>50	>50	50	>50	>50
4	**IV**	50	50	50	>50	>50	>50
5	**V**	>50	50	50	50	>50	>50
6	**VI**	>50	>50	>50	>50	>50	>50
7	**VII**	>50	>50	>50	50	>50	>50
8	**VIII**	>50	>50	>50	>50	>50	>50

Experimental Section:

Melting points were determined in open capillary tubes and are uncorrected. The purity of the compound was checked on silica-gel-coated Al plates (Merck). The structure of the compounds were confirmed on the basis of their Infra red spectra (IR), ^{1}H-NMR, C^{13}NMR and Mass spectra All the compounds gave satisfactory microanalysis. Anti-bacterial and anti-fungal activity screenings were done at CDRI, Lucknow, India.

3-(3,4-Dimethoxyphenyl)-1-(2-hydroxy-5-methylphenyl)prop-2-en-one (I): To a solution of 2-Hydroxy-5-methyl acetophenone (0.1mol) in ethanol (20 ml), 3,4-Dimethoxy benzaldehyde (0.1mol) was added. To this mixture aq. sodium hydroxide (50%, 10ml) was poured gradually while stirring. The mixture was kept at room temperature for 5-8 hrs. with continuous stirring. The sodium salt of chalcone separated was decomposed by ice-cold HCl (30%). The separated chalcone was filtered, washed with water, dried and recrystallized from absolute ethanol. Shiny orange crystals; Yield: 85.36%; m.p: 132-134^{o} C ; IR(KBr) (cm^{-1}): 1633 [C=O], 2931 [C-H], 3021 [Ar-C-H], 3475 [O-H]; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 2.50 (DMSO), 2.33 (s, 3H, -CH_3), 3.83-3.87 (s, 6H, -OCH_3), 6.87-7.93 (m, 6H, Ar-H), 7.54 (s, 1H, -CH), 8.05 (s, 1H, -CH), 12.56 (s, 1H, -OH); ^{13}C-NMR (300 MHz, DMSO-d_6): 193.5, 160.1, 151.6, 149.0, 145.5, 137.08, 130.2, 127.8, 127.2, 124.2, 120.1, 118.8,117.5, 111.5, 111.3, 55.8, 55.6, 40.33, 20.00; Anal. Calc. for $C_{18}H_{18}O_4$: C 72.48, H 6.04; Found: C 72.56, H 5.95.

2-(3,4-Dimethoxyphenyl)-6-methyl-2,3-dihydro-chromen-4-one (II): **(I)** (0.01 mol) was dissolved in ethanol (20 ml). To this solution conc. H_2SO_4 (10ml) was added gradually while stirring. The mixture was refluxed on water bath for 5-8 hrs. After heating it was kept as such for over night when a crystalline solid separated which was filtered, washed with water, dried and recrystallized from absolute ethanol. Shiny white crystals; Yield: 81.88%; m.p: 98-101^{o} C ; IR(KBr) (cm^{-1}): 1332 [C-O-C], 1615 [C-Cl], 1691 [C=O], 2835 [OCH_3], 2927 [CH_3 (C-H)]; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(in ppm) = 2.49 (DMSO), 2.28 (s, 3H, -CH_3), 2.71-2.71 (s, 1H, C_3H), 2.77 (s, 1H, C_3H), 3.76-3.77 (s, 6H, -OCH_3), 6.95-7.57 (m, 6H, Ar-H).; Anal. Calc. for $C_{18}H_{18}O_4$: C 72.48, H 6.04; Found: C 72.54, H 5.97.

2-(3,4-Dimethoxyphenyl)-3-hydroxy-6-methyl-4H-chromen-4-one (III): **(I)** (0.01 mol) was dissolved in ethanol (20 ml). To this solution NaOH (10ml, 20%) was added gradually while stirring. The mixture was kept at 0^oC in ice bath. To this solution H_2O_2 (5ml, 30%) was added with stirring. Further stirring was continued for 2 hrs in ice and then for 3 hrs at room temp (20^oC). A yellow semi-solid was obtained which was acidified with ice cold acetic acid and kept overnight. Precipitate obtained was filtered, washed with water, dried and recrystallized from acetic acid. Pale yellow crystals; Yield: 77.96%; m.p: 184-186^o C ; IR(KBr) (cm^{-1}): 1265 [C-O-C], 1457 [CH_3-(C-H)], 1516 [Ar C=C], 1611 [C=O], 3452 [OH]; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(in ppm) =2.50 (DMSO), 2.44 (s, 3H, -CH_3), 3.85 (s, 6H, -OCH_3), 7.15-7.87 (m, 6H, Ar-H), 9.40 (s, 1H, OH); Anal. Calc. for $C_{18}H_{16}O_5$: C 69.23, H 5.13; Found: C 69.27, H 5.08.

2-(3,4-Dimethoxyphenyl)-6-methyl-4H-chromen-4-one (IV): **(I)** (0.01 mol) was suspended in DMSO (10 ml) and a crystal of iodine was added to it. The mixture was refluxed for 30 mins. The reaction mixture was then cooled, poured on ice cold water. It was then filtered and washed with 10% sodium thiosulphate solution in order to remove iodine. A crystalline solid separated out, which was dried and recrystallized from absolute ethanol. Yellow crystals; Yield: 82.03%; m.p: 78-79^o C ; IR(KBr) (cm^{-1}): 1332 [C-O-C], 1464 [CH_3-(C-H)], 1618 [Ar (C-C)], 1724 [C=O], 3414 [OH]; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(in ppm) =2.50 (DMSO), 2.28 (s, 3H, -CH_3), 3.73 (s, 6H, -OCH_3), 6.86 (s, 1H, C_3H), 7.06-7.43 (m, 6H, Ar-H), 11.21 (s, 1H, OH); Anal. Calc. for $C_{18}H_{16}O_4$: C 72.97, H 5.40; Found: C 73.04, H 5.46.

2-(3,4-Dimethoxybenzylidene)-5-methylbenzofuran-3(2H)-one (V): **(I)** (0.01 mol) and equimolar quantity of $Hg(OAc)_2$ (0.01 mol) was suspended in pyridine (10 ml) and a crystal of iodine was added to it. The mixture was refluxed for 15 mins. The reaction mixture was then cooled, poured on ice cold water, neutralized with 50% ice cold HCl. It was then filtered, dried and recrystallized from absolute ethanol. Yellow crystals; Yield: 72.88%; m.p: 118-120^o C ; IR(KBr) (cm^{-1}): 767 [mono substituted ring], 1263 [C-O-

C], 1435-1472 [-CH def.], 1635 [Ar (C-C)], 1646 [-C=CH, conj.], 1669-1715 [C=O]; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(in ppm) = 2.50 (DMSO), 2.39 (s, 3H, -CH_3), 3.61-3.67 (s, 6H, -OCH_3), 6.53 (s, 1H, CH), 6.77-7.64 (m, 6H, Ar-H).; Anal. Calc. for $C_{18}H_{16}O_4$: C 72.97, H 5.40; Found: C 73.06, H 5.46.

2-(5-(3,4-Dimethoxyphenyl)-4,5-dihydroisoxazol-3-yl)-4-methylphenol (VI): **(I)** (0.01 mol), hydroxylamine hydrochloride (0.0015 mol) and sodium acetate (0.002 mol) in 15 ml ethanol was refluxed for 1 hour. The reaction mixture was cooled and poured on ice cold water. The separated solid product was filtered, washed with water, dried and recrystallized with ethanol. White solid; Yield: 68.07%; m.p:117-120^{o} C; IR(KBr) (cm^{-1}): 817 [disubstituted ring], 1262[Ar (C-OH)], 1678 [C=O, C=N], 1616 [Ar (C=C)], 2837 [-OCH_3], 2936 [aliphatic C-H], 3421 [O-H]; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(in ppm) = 2.44 (s, 1H, CH_3), 2.50 (DMSO), 3.56 (s, 1H, -C_4H oxazoline ring), 3.66 (s, 1H, -C_4H oxazoline ring), 3.71-3.76 (s, 3H, -OCH_3), 5.08 (s, 1H, -C_5H isoxazoline ring), 6.52-7.55 (m, 6H, Ar-H).; Anal. Calc. for $C_{18}H_{19}NO_4$: C 69.01, H 6.07; Found: C 68.95, H 6.15.

(3,4-Dimethoxyphenyl)-1-(furan-2-yl methyl imino) allyl-4-methylphenol (VII): **(I)** (0.01 mol) and furfuryl amine (0.01 mol) was dissolved in 20 ml ethanol. To this mixture 2-3 drops of conc. H_2SO_4 was added and it was refluxed for 3 hrs. On cooling and dilution with ice cold water, a solid mass separated out. It was recrystallized from ethanol-acetic acid. Yellowish cream solid; Yield: 62.40%; m.p:162-165^{o} C; IR(KBr) (cm^{-1}): 1420 [aliphatic C-H def.], 1517 [alkene C=C, conjugated with C=O], 1638 [C=N and furan ring strect], 3461 (O-H).; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 2.27 (s, 1H, CH_3), 2.50 (DMSO), 3.73 (s, 2H, -CH_2), 3.67-3.69 (s, 6H, -OCH_3), 5.10 (s, 1H, CH=CH), 6.52-6.63 (m, 2H, furan ring), 6.78-7.43 (m, 6H, Ar-H), 7.02 (s, 1H, CH=CH), 7.48 (m, 1H, furan ring), 11.64 (s, 1H, -OH); ^{13}C-NMR (300 MHz, DMSO-d_6):193.4, 158.8, 156.8, 148.8, 148.4, 137.5, 133.3, 132.9, 130.4, 127.7, 125.9, 120.8, 117.9, 111.5, 111.3, 110.1, 78.52, 55.5, 54.1, 19.9; M/z = 135; Anal. Calc. for $C_{23}H_{23}NO_4$: C 73.21, H 6.10; Found: C 73.28, H 6.16.

2-(3-(3,4-Dimethoxyphenyl acryloyl)-4-methylphenyl acetate (VIII): **(I)** (0.01 mol), acetic anhydride (0.1 mole, 10.2 ml) and a pinch of fused sodium acetate were refluxed for 30 mins. The reaction mixture was cooled and poured in ice cold water. The solid separated out was washed several times with water, dried and recrystallized from absolute ethanol. Yellowish white solid; Yield: 73.45%; m.p:113-115^{o} C; IR(KBr) (cm^{-1}): 1263 [Ar (-OH)], 1638 [Ar (C=C)], 1682 [Aliph. (C=C)], 1765 [C=O], 2836 [-OCH_3], 2935 [aliphatic C-H], 3455 cm^{-1} [O-H]; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(in ppm) = 2.34 (s, 1H, CH_3), 2.50 (DMSO), 3.82-3.86 (s, 6H, -OCH_3), 5.10 (s, 1H, -CH), 6.52-7.47 (m, 6H, Ar-H), 7.15 (s, 1H, CH), 7.58 (s, 1H, CH); Anal. Calc. for $C_{20}H_{20}O_5$: C 70.59, H 5.88; Found: C 70.65, H 5.81.

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