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Synthesis and biological screening of a combinatorial library of β-chlorovinyl chalcones as anticancer, anti-inflammatory and antimicrobial agents

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This article is dedicated to my grandfather late Pralhadrao G. Gawande.

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ABSTRACT

A combinatorial library of β-chlorovinyl chalcones (**4**) were synthesized by Claisen–Schmidt condensation reaction. Catalytic reaction of substituted 3-chloro-3-phenyl-propenal (**2**) and 1-(2,4-dimethoxy-phenyl)-ethanone or 1-(4-methoxy-phenyl)-ethanone (**3**) in alkaline conditions furnished the target compound 5-chloro-1-(2,4-dimethoxy-phenyl)-5-phenyl-penta-2,4-dien-1-one (**4**). The synthesized compounds were screened for their biological activity viz. anticancer, anti-inflammatory and antimicrobial activities. Synthesized compounds **4g** and **4h** revealed promising anti-inflammatory activity (66–67% TNF-α and 95–97% IL-6 inhibitory activity at 10 μM). Cytotoxicity of the compounds checked using CCK-8 cell lines and found to be nontoxic to slightly toxic. Furthermore, the anticancer activity (30–40%) was shown by compounds **4d**, **4e**, **4h** and **4b** at 10 μM concentrations against ACHN followed by Calu 1, Panc1, HCT116 and H460 cell lines. Some of the compounds **4d**, **4e**, **4a**, **4i** and **4b** revealed promising antimicrobial activity at MIC 50–100 μg/mL against selected pathogenic bacteria and fungi.

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1. Introduction

Combinatorial organic synthesis is still of great interest because it promises to accelerate the drug discovery process as well as speed up the development of new catalysts.[1,2] The synthesis of unbiased compound libraries offers the advantage of rapidly producing a considerable number of potentially active compounds with diverse structures and thus enabling medicinal chemistry projects to more rapidly identify potentially interesting drug candidates to be further pursued.[3] Cancer is one of the leading causes of death in the present society.[4] Over 1 million cases of cancer occur in the United States annually and cancer-related deaths are estimated to reach 12 million worldwide, by the year 2015.[5] Acute and chronic inflammation and different types of arthritis are the inflammatory disorders, which are a big blow to humanity and continual search for newer non-steroidal anti-inflammatory agents is the only way to fortify against this awful threat.[6] There has been a tremendous burst of new development activity during the 1990s that has culminated in the launch of several new classes of anti-inflammatory drugs such as the leukotriene antagonists, cyclooxygenase 2 (COX-2) inhibitors and tumor necrosis factor (TNF) blockers.[7] Moreover, infectious diseases caused by bacteria, fungi, viruses and parasites are also a major threat to public health, despite tremendous progress in medicinal chemistry. The impact is more acute in developing countries due to nonavailability of desired medicines and emergence of widespread drug resistance.[8]

Chalcone derivatives have received a great deal of attention due to their relatively simple structures and wide variety of pharmacological activities reported for these compounds including anticancer, anti-inflammatory, antioxidant, cytotoxicity, antimicrobial, analgesic, antipyretic, anti-anginal, anti-hepatotoxic, antimalarial and anti-allergic activities. These activities are largely attributed due to the unsaturated ketone moiety.[9] Moreover, introduction of substituents into the two aryl rings also a subject of interest because it leads to useful structure–activity relationship (SAR) conclusions and thus helps to synthesize pharmacologically active chalcones.[10] Many of the combinatorial libraries are synthesized and studied for their biological activities.[11,12]

In the previous communications, we reported the pyrazole chalcones and simple chalcones as anticancer, anti-inflammatory, antioxidant and antimicrobial agents.[13] Here, we report a combinatorial synthesis and biological evaluation of substituted β-chlorovinyl chalcones as anticancer, anti-inflammatory and antimicrobial agents.

2. Results and discussion

2.1. Chemistry

In the present investigation 5-chloro-1-(2,4-dimethoxy-phenyl)-5-phenyl-penta-2,4-dien-1-one (**4**) have been prepared by

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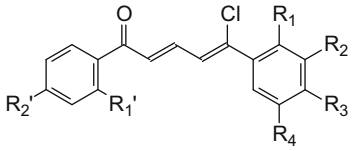
the Claisen–Schmidt condensation of 1-(2,4-dimethoxy-phenyl)-ethanone or 1-(4-methoxy-phenyl)-ethanone (**3**) and substituted 3-chloro-3-phenyl-propenal (**2**) by the known literature method[14] (Scheme 1). Substituted β-chlorovinyl aldehydes (**4**) were synthesized using the method described in the literature with minor modifications.[15] The residue was purified on column chromatography using silica gel with 10% ethyl acetate in hexane. The structures of compounds were confirmed by spectral data (IR, ^{1}H NMR and MS). The chemical profile of the compounds is as shown in Table 1.

Table 1
Structure–analytical data of β-chlorovinyl chalcone derivatives



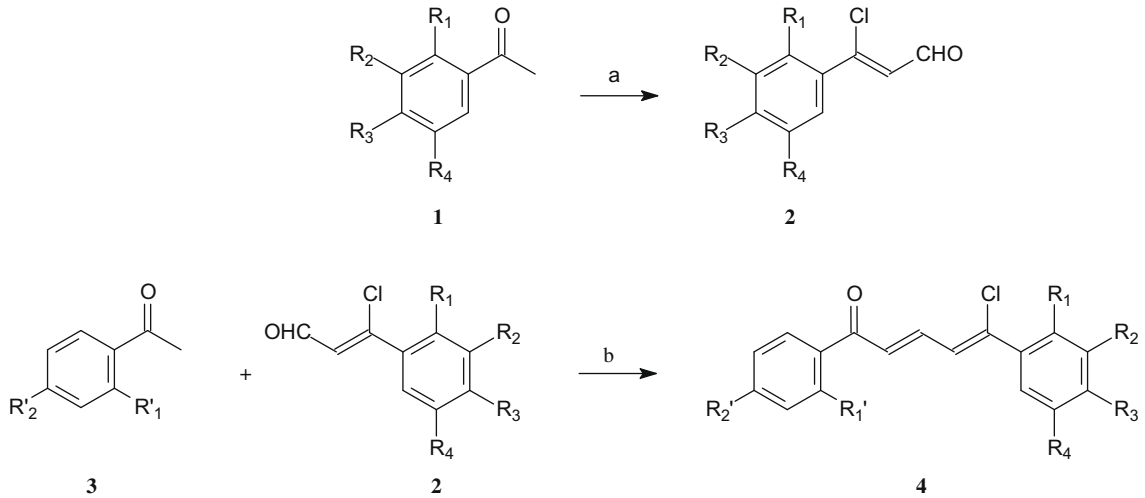
Entry	R'_1	R'_2	R_1	R_2	R_3	R_4	Reaction time (h)	Yield (%)
4a	OCH_3	OCH_3	H	H	Br	H	2	85
4b	OCH_3	OCH_3	H	H	Cl	H	2.2	89
4c	OCH_3	OCH_3	H	H	F	H	2.4	75
4d	OCH_3	OCH_3	H	Cl	H	H	2.1	78
4e	OCH_3	OCH_3	H	H	OCH_3	H	2	91
4f	OCH_3	OCH_3	OCH_3	H	OCH_3	H	3	69
4g	H	OCH_3	H	H	Br	H	2.2	88
4h	H	OCH_3	H	H	Cl	H	2.3	84
4i	H	OCH_3	H	H	F	H	2.5	77
4j	H	OCH_3	H	Cl	H	H	2.9	64
4k	H	OCH_3	H	H	OCH_3	H	2.1	93
4l	H	OCH_3	OCH_3	H	OCH_3	H	2.8	70

2.2. Biological evaluation

Anti-inflammatory activity of the synthesized compounds was determined in terms of TNF-α and IL-6 inhibition. The compounds 4a–**l** does not revealed any acceptable TNF-α inhibitory activity except compounds **4g** and **4h** at 10 μM concentration. As compared to the standard dexamethasone (73% at 1 μM), both the compounds **4g** (66% at 10 μM) and **4h** (67% at 10 μM) equally inhibit the TNF-α activity therefore are potent. All the synthesized compounds were found effective in IL-6 inhibitory activity. Compounds **4g** and **4h** showed promising IL-6 inhibitory activity (95–97% at 10 μM) as compared to standard dexamethasone (84% at 1 μM). Compounds **4e**, **4c**, **4b**, **4l** and **4a** showed moderate activity (36–49% inhibition), while compounds **4f**, **4k**, **4d**, **4i** and **4j** have negligible activity. Cytotoxicity of the compounds was tested for their bioavailability and evaluated using CCK-8 cells. The compounds **4a–f**, **4i** and **4k** were completely nontoxic while **4g**, **4h**, **4j** and **4l** were slightly toxic (Table 2).

Antiproliferative activity of compounds in cell lines from multiple cancer origin is described in Table 3. For the activity, growing cultures of each cell lines were exposed to 10 μM concentration of the different chalcones. In particular, the compounds **4d** and **4e** were active against all the selected cell lines (30–40% inhibition at 10 μM concentration). Further the compounds **4h**, **4b**, **4c** and **4a** (30–39% inhibition at 10 μM concentration) gave moderate inhibitory activity and the remaining compounds (**4g**, **4j**, **4k**, **4l**, **4f** and **4i**) were less effective. Moreover, if the cell lines consider, ACHN was affected more followed by Calu 1, Panc1, HCT116 and H460. The obtained results were found comparable with the standard flavopiridol and gemcitabine.

Antibacterial activity of chalcones is being increasingly documented. Many research groups either isolated or identified the structure of chalcones that possess antibacterial activity of synthesized or modified natural chalcones.[16] The antibacterial activity of the compounds is summarized in Table 4 and found to be comparable with the standard drug tetracycline. MIC_{50} were recorded as the minimum concentration of a compound that inhibits the 50% growth of the tested microorganisms. The compounds **4a**, **4b**, **4d**, **4e**, **4i** and **4j** showed 100% inhibition of all the selected bacterial strains at MIC value of the range of 50 μg/mL, while compounds **4c**, **4k**, **4h**, **4l**, **4g** and **4f** were found to have moderate to negligible antibacterial activity. Out of five selected bacterial strains *Escherichia coli* was affected more followed by *Proteus vulgaris, Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Bacillus subtilis* by all the compounds. The crude mortality from opportunistic fungal infections still exceeds 50% in most human studies and has been reported to be as high as 95% in a bone marrow transplant recipients infected with *Aspergillus* spp.[17] All the tested compounds (**4a**, **4d**, **4e**, **4h**, **4i** and **4k**) illustrated significant antifungal activity as compared to the reference drug nystatin followed by the compounds **4b**, **4g**, **4j** and **4l** at MIC value 100 μg/mL. Compounds **4c** and **4f** inhibit only *Aspergillus flavus* and *Aspergillus niger*, respectively.



Scheme 1. General synthetic route for the synthesis of β-chlorovinyl chalcone derivatives. Reagents and conditions: (a) DMF, $POCl_3$, 0 °C–80 °C, 1 h (b) 40% NaOH, EtOH, 10 °C, 2–3 h.

Table 2
Anti-inflammatory activity of chalcone derivatives

Compounds	% Inhibition at 10 μM		Toxicity
	TNF-α	IL-6	
4a	0	36	0
4b	0	43	0
4c	0	44	0
4d	0	7	0
4e	0	49	0
4f	0	27	0
4g	66	97	30
4h	67	95	28
4i	0	6	2
4j	1	3	35
4k	2	11	0
4l	0	40	35
Dexamethasone (1 μM)	73	84	0

Table 3
Anticancer activity of chalcone derivatives

Compounds	Anticancer activity at 10 μM concn				
	ACHN	Panc1	Calu 1	H460	HCT116
4a	39	33	27	16	22
4b	37	32	32	21	26
4c	30	23	36	26	14
4d	32	32	40	31	37
4e	33	31	31	33	31
4f	26	23	24	18	29
4g	39	25	29	20	20
4h	35	30	32	33	29
4i	17	1	0	14	0
4j	22	18	20	26	31
4k	28	16	38	19	16
4l	33	7	7	15	0
Flavopiridol (700 nM)	68	75	68	84	71
Gemcitabine (500 nM)	70	71	70	68	76

Table 4
Antibacterial activity of β-chlorovinyl chalcones (zone of inhibition in mm)

Compound	Bacteria (MIC at 50 μg/mL)					Fungi (MIC at 100 μg/mL)				
	EC	PV	KN	SA	BS	AN	AF	TV	CA	PC
4a	13	10	18	12	16	12	18	16	12	16
4b	10	18	15	13	14	14	12	12	—	14
4c	11	16	—	—	10	—	10	—	—	—
4d	12	13	16	16	18	14	15	13	10	10
4e	14	15	12	12	20	15	12	14	8	12
4f	—	13	—	—	—	13	—	—	—	—
4g	—	—	11	12	—	—	8	8	—	12
4h	—	—	10	—	—	13	12	12	10	12
4i	11	16	15	16	15	8	15	14	8	15
4j	10	15	17	13	15	—	8	11	—	—
4k	17	—	12	12	—	14	14	14	11	15
4l	10	—	—	—	12	11	10	12	—	—
Tetracycline	15	16	20	18	18	—	—	—	—	—
Nystatin	—	—	—	—	—	13	16	12	11	14
Control	—	±	—	±	—	±	±	±	±	±

Data represent is mean of three replicates.
EC—*Escherichia coli* (MTCC 1650); PV—*Proteus vulgaris* (MTCC 1771); KN—*Klebsiella pneumoniae* (NCIM 2957); SA—*Staphylococcus aureus* (MTCC 96); BS—*Bacillus subtilis* (MTCC 1789); AN—*Aspergillus niger* (MTCC 1781); AF—*Aspergillus flavus* (MTCC 2501); TV—*Trichoderma viridae* (MTCC 167); CA—*Candida albicans* (MTCC 227); PC—*Penicillium chrysogenum* (MTCC 1996); not detected —; trace activity ±.

We also tried to predict that whether the promising activity (anticancer and anti-inflammatory) of **4h** was due to the Cl substitution at fourth position or its due to the enone system of chalcone only (Fig. 1). This can be evaluated by comparing the anticancer and anti-inflammatory activity of the synthesized compounds (**4h**) with that of the commercially available chalcone (**5h**) as summarized in Table 5. The results revealed that the compound **4h** have an increased activity than the regular chalcones (Fig. 2); hence a new chalcone library with enhanced biological activity was confirmed.

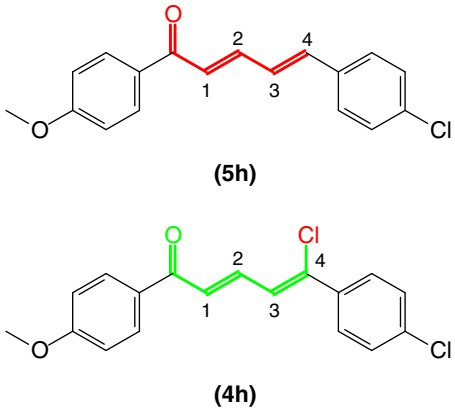


Figure 1. Comparative anticancer and anti-inflammatory activity of **4h** and **5h**.

3. Conclusion

A new series of β-chlorovinyl chalcones exhibiting anticancer, anti-inflammatory and antimicrobial activity was synthesized. From the results of the tested compounds, **4g** and **4h** showed promising activity against TNF-α and IL-6. The promising antiproliferative activity was given by the compounds **4d** and **4e** followed by **4h**, **4b**, **4a** and **4c** as compared to standard drug. ACHN was affected more followed by Calu 1, Panc1, HCT116 and H460. All the compounds revealed good to moderate antimicrobial activity as compared to the reference drug tetracycline and nystatin at MIC 50–100 μg/mL.

4. Experimental

4.1. Synthesis

4.1.1. General procedure for synthesis of substituted β-chlorovinyl aldehyde (2a–f)

Substituted acetophenone **1** (10 mmol) was dissolved in DMF (75 mL) at room temperature and then $POCl_3$ (5.58 mL, 60 mmol) was added dropwise at 0 °C. After complete addition of $POCl_3$, the reaction mixture was warmed to room temperature and then heated at 80 °C for 1 h. The reaction mass was poured onto the crushed ice and then neutralized with a 10% aqueous NaOH solution. The product was extracted with dichloromethane (3 × 100 mL) and extract was washed with water (5–6 times) to remove the excess DMF. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. Finally the crude material was purified by column chromatography using petroleum ether and ethyl acetate (9:1).

4.1.2. Synthesis of β-chlorovinyl chalcones (4a–l)

A mixture of 1-(2,4-dimethoxy-phenyl)-ethanone **3** (0.180 g, 1 mmol) and 3-(4-bromophenyl)-3-chloro-propenal **2a** (0.247 g, 1 mmol) was dissolved in 15 mL ethanol. To this mixture, sodium hydroxide (40%, 2 mL) was added at 0–5 °C. The reaction mixture was stirred at room temperature for 2 h. Then this reaction mixture was poured over crushed ice and acidified with dil HCl. The yellow

Table 5
Comparative study of anticancer and anti-inflammatory activity of **4h** and **5h** compounds

Compounds	Anticancer activity at 10 μM concn					% Inhibition at 10 μM		Toxicity
	ACHN	Panc1	Calu 1	H460	HCT116	TNF-α	IL-6	
4h	35	30	32	33	29	67	95	28
5h	30	24	27	23	24	45	62	32

solid thus obtained was filtered, washed with water and dried. The residue was purified on column chromatography (silica gel with 10% ethyl acetate in hexane) to afford pure 5-(4-bromo-phenyl)-5-chloro-1-(2,4-dimethoxy-phenyl)-penta-2,4-dien-1-one (**4a**) (Scheme 1).

4.2. Physico-chemical data of synthesized compounds

The purified chalcones were obtained in yields of 70–93%. Their structures were identified using infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR) and mass analyses. Melting points were determined with a Microquimica MG APF-301 apparatus and are uncorrected. IR spectra were recorded with a FT Perkin Elmer 16 PC spectrometer on KBr disks. ^{1}H NMR spectra were recorded on a Brucker Ac-200 F (300 MHz) with tetramethylsilane as an internal standard. Mass spectra were obtained on a JMX-HX 100 mass spectrometer. The purity of the synthesized substances was analyzed by thin-layer chromatography (TLC) using Merck silica pre-coated aluminum plates 200 μm in thickness with several solvent systems of different polarities. Compounds were visualized with ultraviolet light (254 nm) and purified on column chromatography (silica gel with 10% ethyl acetate in hexane).

The physical and spectral data of new β-chlorovinyl chalcones (**4a–l**) are given below.

4.2.1. 3-(4-Bromophenyl)-3-chloro-propenal (2a)

Light yellow solid; mp: 67 °C, IR (KBr) 2986, 2731, 1640, 1599, 1210, 1034, 934 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.21 (d, 1H, J = 6.8 Hz), 7.62 (d, 2H, J = 8.8 Hz), 7.60 (d, 2H, J = 8.8 Hz), 6.65 (d, 2H, J = 6.8 Hz). EIMS (70 eV) m/z: 247 (M+1).

4.2.2. 3-(4-Chlorophenyl)-3-chloro-propenal (2b)

Yellow solid; mp: 88 °C, IR (KBr) 2954, 2722, 1641, 1590, 1230, 1023, 915 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.12 (d, 1H, J = 6.8 Hz), 7.71 (d, 2H, J = 8.8 Hz), 7.63 (d, 2H, J = 8.8 Hz), 6.69 (d, 2H, J = 6.8 Hz). EIMS (70 eV) m/z: 201 (M+1).

4.2.3. 3-(4-Fluorophenyl)-3-chloro-propenal (2c)

Colorless solid; mp: 93 °C, IR (KBr) 2918, 2716, 1653, 1600, 1250, 1276, 824 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.26 (d, 1H, J = 6 Hz), 7.81 (m, 2H, J = 8 Hz), 7.62 (d, 2H, J = 8 Hz), 6.69 (d, 2H, J = 6 Hz). EIMS (70 eV) m/z: 185 (M+1).

4.2.4. 3-(3-Chlorophenyl)-3-chloro-propenal (2d)

Light yellow solid; mp: 78 °C, IR (KBr) 2923, 2712, 1643, 1599, 1267, 1056, 756 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.10 (d, 1H, J = 6 Hz), 7.71 (m, 1H), 7.40 (m, 3H), 6.61 (d, 1H, J = 6 Hz). EIMS (70 eV) m/z: 201 (M+1).

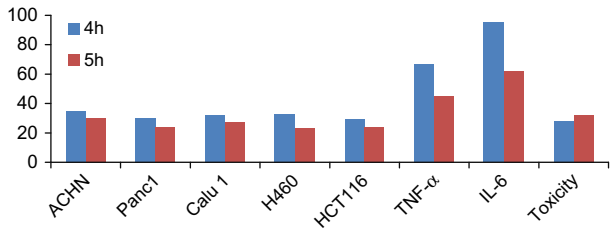


Figure 2. Graphical representation of anticancer and anti-inflammatory activity of **4h** and **5h**.

4.2.5. 3-(4-Methoxyphenyl)-3-chloro-propenal (2e)

Yellow solid; mp: 78 °C, IR (KBr) 2912, 2721, 1656, 1601, 1257, 1278, 829 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.19 (d, 1H, J = 6.8 Hz), 7.72 (d, 2H, J = 8.8 Hz), 6.79 (d, 2H, J = 8.8 Hz), 6.62 (d, 2H, J = 6.8 Hz), 3.89 (s, 3H). EIMS (70 eV) m/z: 197 (M+1).

4.2.6. 3-(2,4-Dimethoxyphenyl)-3-chloro-propenal (2f)

Black solid; mp = 78 °C; IR (KBr) 2943, 2734, 1634, 1589, 1253, 1050, 956 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.173 (d, 1H, J = 6.9 Hz), 7.372 (dd, 1H, J = 2.1 Hz and 2.1 Hz), 7.168 (t, 1H), 6867 (d, 1H, J = 8.6 Hz), 6.573 (d, 1H, J = 6.9 Hz), 3.880 (s, 1H), 3.869 (s, 1H). EIMS (70 eV) m/z: 227 (M+1).

4.2.7. 5-(4-Bromo-phenyl)-5-chloro-1-(2,4-dimethoxy-phenyl)-penta-2,4-dien-1-one (4a)

Light yellow solid; mp = 132 °C; IR (KBr) 3018 (Ar-H), 1610 (C=O), 1591 (C=C) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.79 (dd, 1H, J = 12.6 Hz and 10.4 Hz), 7.57–7.49 (m, 2H), 7.39 (d, 1H, J = 12.6 Hz), 7.24 (s, 1H), 7.19–7.10 (m, 2H), 6.94 (d, 1H, J = 10.4 Hz), 6.54 (d, 1H, J = 8.1 Hz), 6.47 (d, 1H, J = 8.1 Hz), 3.86 (s, 3H), 3.87 (s, 3H). EIMS (70 eV) m/z: 406 (M^+).

4.2.8. 5-Chloro-5-(4-chloro-phenyl)-1-(2,4-dimethoxy-phenyl)-penta-2,4-dien-1-one (4b)

Light yellow solid; mp = 141 °C; IR (KBr) 3010 (Ar-H), 1615 (C=O), 1599 (C=C) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72 (dd, 1H, J = 12.6 Hz and 10.4 Hz), 7.56–7.47 (m, 2H), 7.37 (d, 1H, J = 12.6 Hz), 7.20 (s, 1H), 6.95 (d, 1H, J = 10.4 Hz), 6.55 (d, 2H, J = 8.0 Hz), 6.45 (d, 2H, J = 8.0 Hz), 3.86 (s, 3H), 3.85 (s, 3H). EIMS (70 eV) m/z: 362 (M^+).

4.2.9. 5-Chloro-1-(2,4-dimethoxy-phenyl)-5-(4-fluoro-phenyl)-penta-2,4-dien-1-one (4c)

Yellow solid; mp = 121 °C; IR (KBr) 3025 (Ar-H), 1622 (C=O), 1588 (C=C) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.81 (dd, 1H, J = 12.8 Hz and 10.8 Hz), 7.73 (d, 1H, J = 12.8 Hz), 7.70–7.65 (m, 2H), 7.24 (s, 1H), 7.17–7.03 (m, 2H), 6.88 (d, 1H, J = 10.8 Hz), 6.54 (d, 1H, J = 8.0 Hz), 6.46 (d, 1H, J = 8.0 Hz), 3.88 (s, 3H), 3.86 (s, 3H). EIMS (70 eV) m/z: 346 (M^+).

4.2.10. 5-Chloro-5-(3-chloro-phenyl)-1-(2,4-dimethoxy-phenyl)-penta-2,4-dien-1-one (4d)

Light yellow solid; mp = 113 °C; IR (KBr) 3014 (Ar-H), 1619 (C=O), 1581 (C=C) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.81 (dd, 1H, J = 12.6 Hz and 10.4 Hz), 7.71 (d, 1H, J=12.6 Hz), 7.64 (d, 1H, J = 8.7 Hz), 7.63 (d, 1H, J = 8.7 Hz), 7.24 (s, 1H), 7.14 (d, 1H, J = 10.8 Hz), 6.91–6.87 (m, 2H), 6.56–6.45 (m, 2H), 3.88 (s, 3H), 3.86 (s, 3H). EIMS (70 eV) m/z: 362 (M^+).

4.2.11. 5-Chloro-1-(2,4-dimethoxy-phenyl)-5-(4-methoxy-phenyl)-penta-2,4-dien-1-one (4e)

Light yellow solid; mp = 148 °C; IR (KBr) 3018 (Ar-H), 1616 (C=O), 1594 (C=C) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (dd, 1H, J = 12.3 Hz and 10.8 Hz), 7.72 (d, 1H, J = 12.3 Hz), 7.64 (d, 1H, J = 8.7 Hz), 7.53 (d, 1H, J = 8.7 Hz), 7.24 (s, 1H), 7.10 (d, 1H,

J = 10.8 Hz), 6.90–6.85 (m, 2H), 6.55–6.52 (m, 2H), 3.87 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H). EIMS (70 eV) m/z: 359 (M+1).

4.2.12. 5-Chloro-1,5-bis-(2,4-dimethoxy-phenyl)-penta-2,4-dien-1-one (4f)

Light yellow solid; mp = 123 °C; IR (KBr) 3011 (Ar-H), 1613 (C=O), 1597 (C=C) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.80 (dd, 1H, J = 12.2 Hz and 10.8 Hz), 7.73 (d, 1H, J = 12.2 Hz), 7.63 (d, 1H, J = 8.7 Hz), 7.61 (d, 1H, J = 8.7 Hz), 7.26 (s, 1H), 7.10 (d, 1H, J = 10.8 Hz), 6.90–6.85 (m, 2H), 6.54–6.51 (m, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H). EIMS (70 eV) m/z: 388 (M^+).

4.2.13. 5-(4-Bromo-phenyl)-5-chloro-1-(4-methoxy-phenyl)-penta-2,4-dien-1-one (4g)

Light yellow solid; mp = 132 °C; IR (KBr) 3015 (Ar-H), 1610 (C=O), 1591 (C=C) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.76 (dd, 1H, J = 12.8 Hz and 10.8 Hz), 7.56–7.48 (m, 2H), 7.38 (d, 1H, J = 12.8 Hz), 7.20–7.12 (m, 2H), 6.95 (d, 1H, J = 10.8 Hz), 6.53 (d, 2H, J = 8.0 Hz), 6.46 (d, 2H, J = 8.0 Hz), 3.86 (s, 3H). EIMS (70 eV) m/z: 376 (M^+).

4.2.14. 5-Chloro-5-(4-chloro-phenyl)-1-(4-methoxy-phenyl)-penta-2,4-dien-1-one (4h)

Yellow solid; mp = 138 °C; IR (KBr) 3021 (Ar-H), 1617 (C=O), 1586 (C=C) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.74 (dd, 1H, J = 12.8 Hz and 10.8 Hz), 7.58–7.46 (m, 2H), 7.36 (d, 1H, 12.8 Hz), 7.20–7.15 (m, 2H), 6.90 (d, 1H, J = 10.8 Hz), 6.56 (d, 2H, J = 8.1 Hz), 6.49 (d, 2H, J = 8.1 Hz), 3.86 (s, 3H). EIMS (70 eV) m/z: 332 (M^+).

4.2.15. 5-Chloro-5-(4-fluoro-phenyl)-1-(4-methoxy-phenyl)-penta-2,4-dien-1-one (4i)

Yellow solid; mp = 108 °C; IR (KBr) 3022 (Ar-H), 1623 (C=O), 1596 (C=C) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.81 (dd, 1H, J = 12.8 Hz and 10.8 Hz), 7.73 (d, 1H, J = 12.8 Hz), 7.73 (d, 2H, J = 8.4 Hz), 7.22 (d, 2H, J = 8.4 Hz), 7.02 (d, 2H, J = 8.0 Hz), 6.89 (d, 1H, J = 10.8 Hz), 6.52 (d, 2H, J = 8.0 Hz), 3.86 (s, 3H). EIMS (70 eV) m/z: 316 (M^+).

4.2.16. 5-Chloro-5-(3-chloro-phenyl)-1-(4-methoxy-phenyl)-penta-2,4-dien-1-one (4j)

Light yellow solid; mp = 98 °C; IR (KBr) 3010 (Ar-H), 1616 (C=O), 1586 (C=C) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.82 (dd, 1H, J = 12.7 Hz and 10.8 Hz), 7.72 (d, 1H, J = 12.7 Hz), 7.64 (d, 2H, J = 8.0 Hz), 7.25 (d, 2H, J = 8.0 Hz), 7.15 (d, 2H, J = 10.8 Hz), 6.90–6.87 (m, 2H), 6.56–6.40 (m, 2H), 3.87 (s, 3H). EIMS (70 eV) m/z: 332 (M^+).

4.2.17. 5-Chloro-1,5-bis-(4-methoxy-phenyl)-penta-2,4-dien-1-one (4k)

Light yellow solid; mp = 152 °C; IR (KBr) 3025 (Ar-H), 1629 (C=O), 1594 (C=C) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.81 (dd, 1H, J = 12.8 Hz and 10.8 Hz), 7.70 (d, 1H, J = 12.8 Hz), 7.64 (d, 2H, J = 8.7 Hz), 7.20 (s, 1H), 7.12 (d, 1H, J = 10.8 Hz), 6.75–6.60 (m, 2H), 6.40–6.56 (m, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H). EIMS (70 eV) m/z: 329 (M+1).

4.2.18. 5-Chloro-5-(2,4-dimethoxy-phenyl)-1-(4-methoxy-phenyl)-penta-2,4-dien-1-one (4l)

Light yellow solid; mp = 129 °C; IR (KBr) 3012 (Ar-H), 1617 (C=O), 1592 (C=C) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.81 (dd, 1H, J = 12.8 Hz and 10.8 Hz), 7.70 (d, 1H, J = 12.8 Hz), 7.64 (d, 2H, J = 8.7 Hz), 7.20 (s, 1H), 7.12 (d, 1H, J = 10.8 Hz), 6.75–6.60 (m, 2H), 6.40–6.56 (m, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H). EIMS (70 eV) m/z: 358 (M^+).

4.3. Anti-inflammatory and cytotoxicity assay

Proinflammatory cytokine production by lipopolysaccharide (LPS) in THP-1 cells was measured according to the method described by Hwang et al.[18] During assay, THP-1 cells were cultured in RPMI 1640 culture medium (Gibco BRL, Pasley, UK) containing 100 U/mL penicillin and 100 mg/mL streptomycin containing 10% fetal bovine serum (FBS, JRH). Cells were differentiated with phorbol myristate acetate (PMA, Sigma). Following cell plating, the test compounds in 0.5% DMSO were added to each well separately and the plate was incubated for 30 min at 37 °C. Finally, LPS (*E. coli* 0127:B8, Sigma Chemical Co., St. Louis, MO) was added, at a final concentration of 1 μg/mL in each well. Plates were further incubated at 37 °C for 24 h in 5% CO_2. After incubation, supernatants were harvested, and assayed for TNF-α and IL-6 by ELISA as described by the manufacturer (BD Biosciences). The cells were simultaneously evaluated for cytotoxicity using CCK-8 from Dojindo Laboratories. Percent inhibition of cytokine release compared to the control was calculated. The 50% inhibitory concentration (IC_{50}) values were calculated by a nonlinear regression method.

4.4. Anticancer activity

Cytotoxic assay is performed on ACHN (human renal cell carcinoma), Panc1 (human pancreatic carcinoma), Calu 1 (human non-small cell lung carcinoma), H460 (human non-cell lung carcinoma) and HCT116 (human colon carcinoma) cell line using propidium iodide fluorescence assay.[19] Dyes such as propidium iodide (PI), which bind DNA, provide a rapid and accurate means for quantitating total nuclear DNA. The fluorescence signal intensity of the PI is directly proportional to the amount of DNA in each cell, PI is not able to penetrate an intact membrane, and so cells must first be permeabilized. Seed cells of 3000–7500 cells/well were placed in 2000 μL of tissue culture grade 96-well plates and allowed them to recover for 24 h in humidified 5% CO_2 incubator at 37 °C. After culturing for 24 h compounds (in 0.1% DMSO) were added onto triplicate wells with 10 μM concentrations. 0.1% DMSO alone was used as control. After 48 h in humidified 5% CO_2 incubator at 37 °C condition, the medium was removed and treated with 25 μL of propidium iodide (50 μg/mL in water/medium) per well. The plates were freeze at −80 °C for 24 h then thawed and allowed it to come at room temperature and the plate absorbance was read on fluorometer (Polar-Star BMG Tech), using 530 nm excitation and 620 nm emission wavelength. Lastly percent cytotoxicity of the compounds was calculated by using following formula.

$$\text{Percent cytotoxicity} = \frac{\text{Reading of control} - \text{Reading of treated cells}}{\text{Reading of control}} \times 100$$

The results were compared with the standard drug inhibitors flavopiridol (700 nM) and gemcitabine (500 nM).

4.5. Antimicrobial activity (agar diffusion method)

Antimicrobial activity of all synthesized compounds was determined by agar diffusion method.[20,21] All human pathogenic bacteria viz. *B. subtilis* (MTCC 1789), *K. pneumoniae* (NCIM 2957), *S. aureus* (MTCC 96), *P. vulgaris* (MTCC 1771), *E. coli* (MTCC 1650) and fungi viz. *Trichoderma viridae* (MTCC 167), *A. flavus* (MTCC 2501), *A. niger* (MTCC 1781), *Candida albicans* (MTCC 227) and

Penicillium chrysogenum (MTCC 1996) were procured from Institute of Microbial Technology (IMTech), Chandigarh, India and National Collection of Industrial Microorganisms (NCIM), Pune, India. Stock solutions of compounds were diluted in dimethyl sulfoxide (1% DMSO) to give final concentration ranging from 25 to 250 μg/mL for determining the MIC value. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of compound required for complete inhibition of the fungal and bacterial growth after incubation time. For antifungal activity, different fungal spore suspensions in sterile distilled water were adjusted to give a final concentration of 10^6 cfu/mL. An inoculum of 0.1 mL spore suspension of each fungus was spreaded on Sabouraud's Dextrose agar plates (HiMedia). For antibacterial activity Muller Hinton agar was used (HiMedia) seeded with 0.1 mL of respective bacterial culture strains suspension prepared in sterile saline (0.85%) of 10^5 cfu/mL dilution. The wells of 6 mm diameter were filled with 0.1 mL of each compound dilution separately for each test of fungi and bacterial strain. The DMSO (1%) alone was used as control. The antibiotics nystatin (30 μg/mL) and tetracycline (10 μg/mL) are used as reference antifungal and antibacterial agent, respectively, for comparison. Inoculated plates in duplicate were then incubated at 37 ± 0.5 °C for antibacterial activity for 24 h and at 28 ± 0.2 °C for antifungal activity for 48 h. After incubation the antimicrobial activity was measured in terms of the zone of inhibition in mm as shown in Table 4.

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