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Synthesis and biological evaluation of simple methoxylated chalcones as anticancer, anti-inflammatory and antioxidant agents

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Dedicated to my research colleague late Ms. Sunita B. Bandgar

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ABSTRACT

Chalcones have been identified as interesting compounds with cytotoxicity, anti-inflammatory and antioxidant properties. In the present study, simple methoxychalcones were synthesized by Claisen–Schmidt condensation reaction and evaluated for above biological activities. The structures of the compounds were established by IR, ^{1}H NMR and mass spectral analysis. The data revealed that compound **3s** (99–100% at 10 μM concentration) completely inhibit the selected five human cancer cell lines as compared to standard flavopiridol and gemcitabine (70–90% at 700 nM and 500 nM concentrations, respectively), followed by **3a**, **3n, 3o, 3p, 3q, 3r**. Among the tested compounds **3l**, **3m**, **3r**, and **3s** exhibited promising anti-inflammatory activity against TNF-α and IL-6 with 90–100% inhibition at 10 μM concentration. DPPH free radical scavenging activity was given by the compounds **3o**, **3n**, **3l**, **3r**, **3m**, **3a**, **3p**, **3c** and **3s** at 1 mM concentration. Overall, **3s** was obtained as lead compound with promising anticancer, anti-inflammatory and antioxidant activities. Bioavailability of compounds were checked by in vitro cytotoxicity study and confirmed to be nontoxic. The structure activity relationship (SAR) and in silico drug relevant properties (HBDs, HBAs, PSA, *c* Log *P*, ionization potential, molecular weight, E_{HOMO} and E_{LUMO}) further confirmed that the compounds were potential candidates for future drug discovery study.

1. Introduction

Chalcones (1,3-diaryl-2-propen-1-ones) constitute an important class of natural products belonging to the flavonoids family, display interesting biological activities including anticancer, anti-inflammatory, antioxidant, cytotoxic, antimicrobial, analgesic and antipyretic, anti-anginal, anti-hepatotoxic, antimalarial and anti-allergic. Chemically they consist of open-chain flavonoids in which the two aromatic rings are joined by a three carbon α,β-unsaturated carbonyl system.[1,2] Cancer, the uncontrolled, rapid and pathological proliferation of abnormal cells, is the second leading cause of human death after cardiovascular diseases in developing as well as advanced countries.[3,4] Although there are many therapeutic strategies including chemotherapy and radiotherapy, high systemic toxicity and drug resistance limit the successful outcomes in most cases. Therefore, novel diagnosis, treatment and prevention approaches are urgently needed for cancer therapy.[5]

Among the naturally occurring hydroxy chalcones and their synthetic analogues, several compounds displayed cytotoxic activity (antimitotic, cell growth inhibitor) towards cultured tumor cells. They are also effective in vivo as cell proliferating inhibitors, anti-tumor promoting and chemopreventing agents. Since a number of clinically useful anticancer drugs have genotoxic effects due to interaction with the amino groups of nucleic acids, chalcones may be devoid of this important side effect.[6–8]

Interleukin-6 (IL-6) and Tumor necrosis factor alpha (TNF-α) are two important multifunctional proinflammatory cytokines involved in pathogenesis of cardiovascular, neurodegenerational diseases and cancer through a series of cytokine signaling pathways.[9] Recently it is also suggested that tumor necrosis factor alpha (TNF-α) is act target for cancer therapy.[10] Literature survey described dimethoxy and trimethoxychalcone derivatives as effective anti-inflammatory agents.[11–13] A number of therapeutically useful NSAID's have been shown to act by virtue of their free radical scavenging activity, which is implicated in the induction and prolongation of inflammatory process.[14]

Reactive oxygen species (ROS) in the form of superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) attack various biological macromolecules (proteins, enzymes, DNA, etc.) under 'oxidative stress' conditions, give rise to a number of inflammatory, metabolic disorders, cellular aging, reperfusion damage and cancer.[15,16] Cytotoxic effects of antioxidant flavonoids (including chalcones) are associated with their pro-oxidant effects.[8] The

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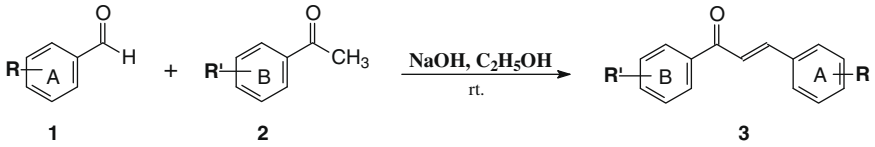


Scheme 1.

antioxidant properties of chalcones are known to be influenced to a great extent by the two aryl structures, that is, the substitutions on two aryl rings of chalcone molecule and their substitution patterns. Especially, the hydroxyl substituent is one of the key groups to enhance greatly the antioxidant activity of chalcone mainly due to its easy conversion to phenoxy radicals through the hydrogen atom transfer mechanism.[17]

In continuation of our research work, in order to find the broad spectrum biologically active chalcones, we have previously described the anti-inflammatory, antimicrobial and antioxidant activity of pyrazole chalcones.[18] Here, we report the synthesis and biological activity of methoxychalcones as an anticancer, anti-inflammatory and antioxidant agents. In the structure activity relationship (SAR) studies, the biological properties of these molecules were compared with several theoretical parameters such as E_{HOMO}, E_{LUMO}, *c* Log *P*, PSA, ionization potential, molecular weight, hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD), calculated using computational softwares. Since the compounds are considered for oral delivery, they were also submitted to the analysis of Lipinski rule of five.[19] Finally, toxicity of compounds was experimentally and theoretically evaluated to determine their potential as safe leading compounds.

2. Results and discussion

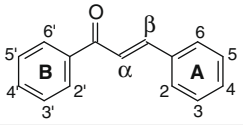
2.1. Chemistry

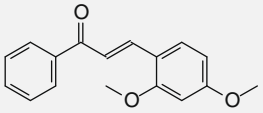
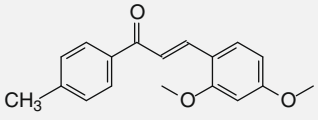
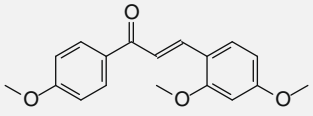
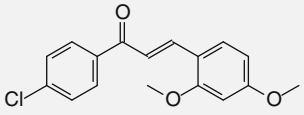
In the present investigation substituted 3-(2,4-dimethoxy-phenyl)-1-phenyl-propenone or 1-phenyl-3-(3,4,5-trimethoxy-phenyl)-propenone (**3**) have been prepared by the Claisen–Schmidt condensation of substituted 1-phenyl-ethanone (**2**) and 2,4-dimethoxy-benzaldehyde or 3,4,5-trimethoxy-benzaldehyde (**1**) by known literature method[18] (Scheme 1). The residue was purified on column chromatography using silica gel with 10% ethyl acetate in hexane. The products were characterized by comparison of their spectral and physical data with those of authentic samples. All authentic chalcones were prepared from the corresponding reactants according to the method described.[20] The chemical profile of the compounds is as shown in Table 1.

2.2. Biological evaluation

Among the currently identified anti-tumor agents, chalcones represents an important class of molecules that are abundant in edible plants (fruits and vegetables). The anticancer activity of certain chalcones is believed to be a result of binding to tubulin and preventing it from polymerizing into microtubules.[21,22] Here, we synthesized 2,4-dimethoxy (**3a–k**) and 3,4,5-trimethoxy (**3l–s**) chalcone derivatives and evaluated for their anticancer activity against the five human cancer cell lines including ACHN (renal cell carcinoma), Pancc1 (pancreatic carcinoma), Calu1 (non-small cell lung carcinoma), H460 (non-small cell lung carcinoma), and HCT116 (colon carcinoma). 2,4-Dimethoxychalcone (**3a**) gives promising anticancer activity with 90–95% of cell line inhibition. Substitution pattern in B-ring was changed simultaneously with CH_3, OCH_3, Cl, Br, F and NO_2 groups (**3b–k**), showed only moderate cell lines inhibition (15–50%) at 10 μM concentration as per Table 2. Introduction of one more methoxy group in A-ring (3,4,5-tri-

Table 1
Synthesis of simple methoxylated chalcones



S.NO.	B-ring			A-ring				Product (3)	Yield[a] (%)	Reaction time (min)
	2′-	3′-	4′	2-	3-	4-	5-			
a	H	H	H	OCH_3	H	OCH_3	H		91	35
b	H	H	CH_3	OCH_3	H	OCH_3	H		96	30
c	H	H	OCH_3	OCH_3	H	OCH_3	H		86	45
d	H	H	Cl	OCH_3	H	OCH_3	H		90	48

(*continued on next page*)

Table 1 (*continued*)

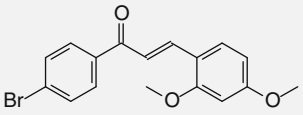
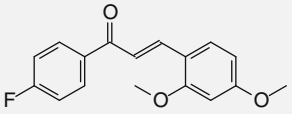
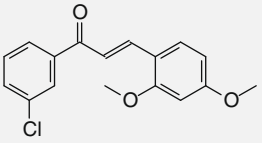
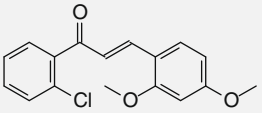
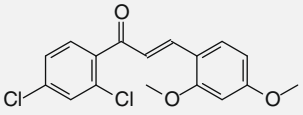
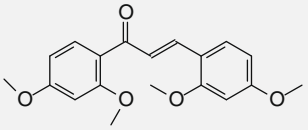
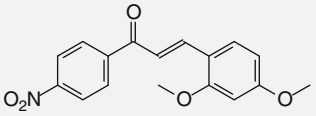
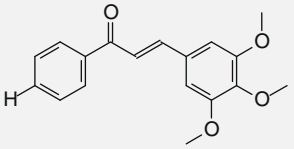
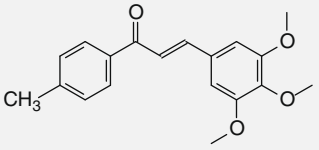
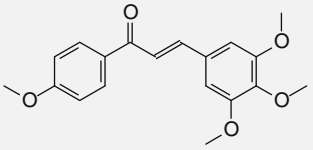
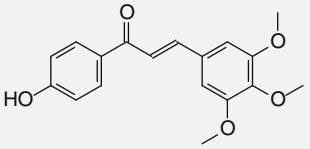
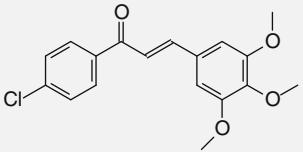
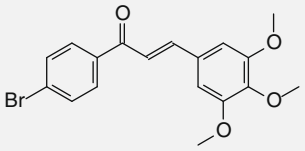
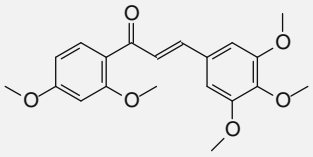
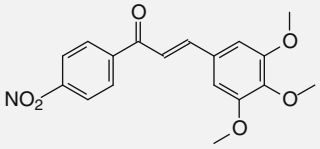
S.NO.	B-ring			A-ring				Product (3)	Yield[a] (%)	Reaction time (min)
	2′-	3′-	4′	2-	3-	4-	5-			
e	H	H	Br	OCH_3	H	OCH_3	H		94	32
f	H	H	F	OCH_3	H	OCH_3	H		96	45
g	H	Cl	H	OCH_3	H	OCH_3	H		84	60
h	Cl	H	H	OCH_3	H	OCH_3	H		80	70
i	Cl	H	Cl	OCH_3	H	OCH_3	H		92	45
j	OCH_3	H	OCH_3	OCH_3	H	OCH_3	H		94	75
k	H	H	NO_2	OCH_3	H	OCH_3	H		95	25
l	H	H	H	H	OCH_3	OCH_3	OCH_3		96	30
m	H	H	CH_3	H	OCH_3	OCH_3	OCH_3		91	40
n	H	H	OCH_3	H	OCH_3	OCH_3	OCH_3		89	55
o	H	H	OH	H	OCH_3	OCH_3	OCH_3		85	50
p	H	H	Cl	H	OCH_3	OCH_3	OCH_3		92	30

Table 1 (*continued*)

S.NO.	B-ring			A-ring				Product (3)	Yield[a] (%)	Reaction time (min)
	2′-	3′-	4′	2-	3-	4-	5-			
q	H	H	Br	H	OCH_3	OCH_3	OCH_3		95	25
r	OCH_3	H	OCH_3	H	OCH_3	OCH_3	OCH_3		94	75
s	H	H	NO_2	H	OCH_3	OCH_3	OCH_3		85	45

[a] Isolated yield.

methoxychalcone), **3l** shows negligible anticancer activity. Further substitution of NO_2 in B-ring (**3s**) increases the inhibitory activity up to 100% as compared to the standard flavopiridol (700 nM) and gemcitabine (500 nM). It is reported that some nitro compounds are afraid to may possess carcinogenicity and mutagenicity.[23] This found to be in contrast in case of compound (**3s**), which is revealed potent anticancer agent without inducing any drug toxicity. Remaining compounds **3n**, **3o**, **3p**, **3q** and **3r** substituted with methoxy, hydroxyl and halo groups showed considerable anticancer activity (50–95%) in the order of OCH_3 > OH > Cl > Br. Edwards et al. confirmed that, more the methoxy groups in a compound, is beneficial for antimitotic activity against HeLa cells.[24] This found contrast in case of compound (**3r**), which might be due to the more bulky nature of the compound.[22] Fluorinated organic molecules are known to perform a wide rang of biological functions and fluorinated anticancer agents have become a focus in the development of new therapies for cancer.[25] Although compound (**3f**) is with fluorine, it doesn't show any remarkable anticancer activity. In general, cell line HCT116 was affected more by all the compounds followed by Pancc1 > ACHN > Calu1 > H460 cell lines (Table 2).

Non-steroidal anti-inflammatory drugs (NSAIDs) are therapeutically important in the treatment of rheumatic arthritis and various types of inflammatory conditions but found to be limited because of their frequently observed gastrointestinal side effects.[14] Being a natural origin, chalcones may be devoid of toxicity and hence beneficial for drug discovery as active compound.[21] Anti-inflammatory activity of all the synthesized compounds was evaluated in terms of TNF-α and IL-6 inhibitory activity. Entire 3,4,5-trimethoxychalcones (**3l–s**) inhibit 90–100% at 10 μM concentration as compared to standard dexamethasone and 2,4-dimethoxychalcones (**3a–k**). Compounds **3l**, **3m**, **3r**, and **3s** found to be promising anti-inflammatory agents without toxicity (Table 3). Interestingly, several studies have demonstrated endogenous TNF-α as a tumor promoter and metastatic factor.[31–33] Sig-

Table 2
Anticancer activity of chalcone derivatives

Compounds	Anti-cancer activity at 10 μM concd				
	ACHN	Panc1	Calu 1	H460	HCT116
3a	93	94	91	92	95
3b	44	45	37	34	31
3c	45	36	31	38	37
3d	28	29	28	29	22
3e	25	30	21	32	32
3f	35	36	30	35	37
3g	36	36	37	22	27
3h	30	37	38	26	25
3i	35	35	30	29	26
3j	48	43	32	38	31
3k	28	16	38	19	16
3l	33	7	7	15	0
3m	17	1	0	14	0
3n	92	93	93	89	97
3o	83	76	76	74	94
3p	57	66	67	54	92
3q	48	57	57	44	86
3r	79	62	58	57	84
3s	99	100	100	100	99
Flavopiridol (700 nM)	68	75	68	84	71
Gemcitabine (500 nM)	70	71	70	68	76

Table 3
Anti-inflammatory and % antioxidant activity of chalcone derivatives

Compounds	% Inhibition at 10 μM		% Antioxidant activity[b] (1 mM/mL)	Toxicity
	TNF-α	IL-6		
3a	98	100	38.00 ± 0.24	79
3b	0	29	25.27 ± 0.22	16
3c	0	39	32.33 ± 0.25	29
3d	0	12	26.39 ± 0.15	0
3e	0	0	0	15
3f	20	61	0	45
3g	0	27	19.21 ± 0.19	0
3h	0	18	10.12 ± 0.27	0
3i	0	6	11.28 ± 0.22	2
3j	2	81	31.32 ± 0.29	35
3k	0	0	18.23 ± 0.29	0
3l	100	100	47.23 ± 0.46	73
3m	97	99	43.21 ± 0.66	29
3n	100	100	48.29 ± 0.45	89
3o	99	100	52.00 ± 0.43	70
3p	98	100	36.53 ± 0.32	79
3q	99	100	23.32 ± 0.23	68
3r	99	100	44.91 ± 0.33	39
3s	65	93	30.21 ± 0.25	18
Dexamethasone (1 μM)	73	84		0
BHA [a]			74.00 ± 0.53	

[a] Standard substance.
[b] Mean ± SD, $n = 3$.

Table 4
In silico pharmacological parameters for bioavailability

Compounds	Molecular formula	Molecular Weight	Mp (°C)	E_{HOMO}	E_{LUMO}	Ionization potential (eV)	HBD	HBA	PSA (A^2)	*c* log *P*	Drug likeness	Drug score
3a	$C_{17}H_{16}O_3$	268.313	60	−9.092	−0.453	8.987	0	3	29.71	3.3	−0.48	0.33
3b	$C_{18}H_{18}O_3$	282.342	74	−9.396	−0.458	9.354	0	3	29.71	3.61	−0.75	0.29
3c	$C_{18}H_{18}O_4$	298.341	82	−8.877	−0.358	8.879	0	4	38.1	3.19	0.14	0.36
3d	$C_{17}H_{15}O_3Cl$	302.757	120	−9.47	−0.636	9.452	0	3	29.71	3.91	2.12	0.38
3e	$C_{17}H_{15}O_3Br$	347.208	152	−9.125	−0.725	9.408	0	3	29.71	4	−2.97	0.2
3f	$C_{17}H_{15}O_3F$	286.302	136	−9.182	−0.555	9.515	0	3	29.71	3.36	0.58	0.37
3g	$C_{17}H_{15}O_3Cl$	302.757	86	−9.184	−0.609	9.219	0	3	29.71	3.91	−1.87	0.23
3h	$C_{17}H_{15}O_3Cl$	302.757	79	−9.121	−0.564	9.133	0	3	29.71	3.91	−0.07	0.5
3i	$C_{17}H_{14}O_3Cl_2$	337.202	125	−9.179	−0.65	9.192	0	3	29.71	4.52	0.96	0.46
3j	$C_{19}H_{20}O_5$	328.364	148	−9.057	−0.292	9.087	0	5	46.23	3.09	0.91	0.5
3k	$C_{17}H_{15}NO_5$	315.325	134	−9.153	−0.651	9.129	0	5	63.11	3.31	−0.53	0.14
3l	$C_{18}H_{18}O_4$	313.311	173	−9.118	−0.957	8.711	0	4	37.62	3.19	4	0.47
3m	$C_{19}H_{20}O_4$	312.365	122	−9.124	−0.917	8.697	0	4	37.62	3.51	3.95	0.43
3n	$C_{19}H_{20}O_5$	328.364	129	−9.141	−0.921	8.703	0	5	46.01	3.09	4.81	0.46
3o	$C_{18}H_{18}O_5$	314.337	98	−9.069	−0.947	8.714	1	5	54.88	2.89	5.19	0.49
3p	$C_{18}H_{17}O_4Cl$	332.783	105	−9.221	−1.05	8.753	0	4	37.62	3.81	6.51	0.4
3q	$C_{18}H_{17}O_4Br$	377.234	102	−9.174	−1.078	8.771	0	4	37.62	3.89	1.74	0.35
3r	$C_{20}H_{22}O_6$	358.39	127	−9.117	−0.696	9.081	0	6	54.14	2.89	3.11	0.74
3s	$C_{18}H_{17}NO_6$	343.351	136	−9.077	−0.97	9.157	0	6	71.02	3.2	4.08	0.21

nificant levels of TNF-α was found in tumor microenvironment of various human cancers, including those of breast, ovarian, prostate, lymphoma, melanoma and leukemia.[34] We demonstrated that TNF-α libration in human THP-1 cells was inhibited by synthesized compounds .This effect revealed that they have dual activity as anti-inflammatory as well as more effective against cancer treatment since, it can act by two mechanisms, directly by killing tumor cells and indirectly, resolving the inflammatory environment that supports tumor development.

Reactive oxygen species and nitrogen specie contribute to the pathophysiology of anti-inflammatory conditions.[16] Antioxidant are the compounds capable of scavenging the free radicals; for this antioxidant therapy is one of the recent options.[26] The antioxidant activity of the compounds was determined by DPPH free radical scavenging activity. Free radical scavenging activity was measured in terms of %antioxidant activity as represented in Table 3. Compounds **3o**, **3l**, **3n**, **3r**, **3p**, **3m**, **3a**, **3c** and **3s** showed 30–55% inhibition as compared to standard BHA (74%). A moderate antioxidant activity of the compound is related with their electron or hydrogen radical releasing ability to DPPH so that it became stable diamagnetic molecule. Hydroxy and methoxy groups are more electron releasing atoms, therefore induces more antioxidant activity.[17] Bioavailability of the compounds was checked by in vitro cytotoxicity assay using CCK-8 cells in RPMI 1640 culture media. Dimethoxylated chalcones (**3a–k**) were found to be nontoxic as compared to trimethoxylated chalcones (**3l–s**), except **3l**, **3m**, **3r** and **3s**. All the synthesized compounds were also tested by Lipinski rule of five to find new potential drug candidates. The results reveled that all compounds are within the range set by Lipinski rule of five as per Table 4. These data have significance for the biological activity for interaction with the receptor/enzyme, penetration through the cell membrane, chemical properties of the molecule during drug metabolism.[27]

3. Conclusion

In summary, we have prepared and evaluated a series of 2,4-dimethoxy and 3,4,5-trimethoxy chalcones for their biological activities. 3,4,5-Trimethoxychalcones inhibit the growth of five human cancer cell lines. Some of them are exciting, as they preferentially inhibit the cell lines more or similar with the standard anticancer agents (flavopiridol and gemcitabine). Moreover promising anti-inflammatory and antioxidant activities were also obtained by the same compounds, which is beneficial for cancer treatment also. Bioavailability and toxicity of the compounds revealed the compounds to be nontoxic with drug properties. The results of this study may find a lead (**3s**) toward the development of new therapeutic agent to fight cancer.

4. Experimental

4.1. General

Melting points were recorded in open capillaries with electrical melting point apparatus and were uncorrected. IR spectra (KBr disks) were recorded using a Perkin–Elmer 237 spectrophotometer. ^{1}H NMR spectra were recorded on Bruker Avance (400 MHz) Spectrometer in $CDCl_3$ solutions, with TMS as an internal reference. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX. All the reagents and solvents used were of analytical grade and were used as supplied unless otherwise stated. TLC was performed on silica gel coated plates for monitoring the reactions.

4.2. Synthesis of chalcones (3a–s)

A mixture of 1-(4-methoxy-phenyl)-ethanone **2** (0.150 g, 1 mmol) and 2,4-dimethoxy-benzaldehyde **1** (0.166 g, 1 mmol) was dissolved in 10 mL ethanol. To this mixture, sodium hydroxide (40%, 1 mL) was added at 0–5 °C. The reaction mixture was stirred at room temperature for 45 min. Then this reaction mixture was poured over crushed ice and acidified with dil HCl. The light yellow 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 3-(2,4-dimethoxy-phenyl)-1-(4-methoxy-phenyl)-propenone (**3c**) (Scheme 1).

The physical and spectral data of selective methoxylated chalcones are given below.

4.2.1. 3-(2,4-Dimethoxy-phenyl)-1-(4-methoxy-phenyl)-propenone (3c)

Pale yellow solid, mp 82 °C, IR (KBr disk): 3062, 2933, 1644, 1699, 1279 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (d, J = 15.6 Hz, 1H), 8.03 (d, J = 8.6 Hz, 2H), 7.55 (d, J = 15.6 Hz, 1H), 7.57 (d, J = 8.6 Hz, 1H), 6.96 (d, J = 8.6 Hz, 2H), 6.53 (dd, J_1 = 8.4, J_2 = 2.3 Hz, 1H), 6.47 (d, J = 2.3 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H); MS (ESI) m/z = 299 (M+1).

4.2.2. 1-(4-Bromo-phenyl)-3-(2,4-dimethoxy-phenyl)-propenone (3e)

Light Yellow, mp: 152 °C, IR (KBr) 2920, 2827, 1648, 1597, 1443, 1357, 1263, 1157, 1022, 813 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.95 (d, J = 7.1 Hz, 2H), 7.67 (d, J = 15.5 Hz, 1H), 7.52 (d, J = 15.5 Hz, 1H), 7.24 (d, J = 8.4 Hz, 1H), 7.22 (s, 1H), 6.93 (d, J = 8.4 Hz, 1H), 6.87 (d, J = 7.1 Hz, 2H), 3.91 (s, 3H), 3.87 (s, 3H); MS (ESI) m/z = 347 (M+1).

4.2.3. 1-(2,4-Dichloro-phenyl)-3-(2,4-dimethoxy-phenyl)-propenone (3i)

Yellow solid, mp: 125 °C, IR (KBr) 2968, 2832, 1653, 1512, 1469, 1315, 1262, 1142, 1025, 801 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.10 (s, 1H), 7.85 (m, 1H), 7.79 (d, J = 15.6 Hz, 1H), 7.59 (d, J = 8.3 Hz, 1H), 7.30 (d, J = 15.6 Hz, 1H), 7.26 (d, J = 8.9 Hz, 1H), 7.17 (s, 1H), 6.91 (d, J = 8.3 Hz, 1H), 3.97 (s, 3H), 3.95 (s, 3H); MS (ESI) m/z = 339.1 (M+2).

4.2.4. 3-(2,4-Dimethoxy-phenyl)-1-(4-nitro-phenyl)-propenone (3k)

Yellow solid, mp: 134 °C, IR (KBr) 2930, 1650, 1523, 1342, 1257, 1161, 1016, 905 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, J = 15.6 Hz, 1H), 7.97 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 8.6 Hz, 1H), 7.70–7.52 (m, 3H), 7.37 (d, J = 15.6 Hz, 1H), 6.58 (dd, J = 8.6, 2.1 Hz, 1H), 6.50 (d, J = 2.1 Hz, 1H), 3.90 (s, 3H), 3.88 (s, 3H); MS (ESI) m/z = 315.1 (M+1).

4.2.5. 1-Phenyl-3-(3,4,5-trimethoxy-phenyl)-propenone (3l)

Light yellow solid, mp: 173 °C, IR (KBr disk): 3069, 2932, 1640, 1696, 1272 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.90 (d, J = 16.4 Hz, 1H), 7.81 (d, J = 8.2 Hz, 2H), 7.56 (d, J = 16.4 Hz, 1H), 7.57 (d, J = 8.6 Hz, 1H), 6.96 (d, J = 8.6 Hz, 2H), 6.50 (dd, J_1 = 8.4, J_2 = 2.3 Hz, 1H), 6.45 (d, J = 2.3 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H); MS (ESI) m/z = 299 (M+1).

4.3. Anticancer activity

Cytotoxic assay is performed on ACHN (human renal cell carcinoma), Panc 1 (human pancreatic carcinoma), Calu 1 (human non small cell lung carcinoma), H460 (human non cell lung carcinoma) and HCT 116 (human colon carcinoma) cell line using propidium iodide fluorescence assay.[28] 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 permeabilised. 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).

4.4. 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.[29] 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 and the plate was incubated for 30 min at 37 °C. Finally, LPS (*Escherichia 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.

4.5. In vitro antioxidant activity (DPPH method)

The compounds (**3a**–**s**) were evaluated for their in vitro free radical scavenging activity by the 2,2′diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method described by Blois .[30] Stock solutions of different compounds (1 mM) were mixed with DPPH methanol solution (0.5 mL, 0.3 mM) in 3 mL of total reaction mixture and allowed to react at room temperature. After 30 min, absorbance values were measured at 520 nm and converted to %antioxidant activity. For a comparative study the Butylated hydroxyl anisole (BHA) was used as the standard. The percentage inhibition activity was calculated by using a formula.

$$\% \text{ Activity} = [1 - \text{OD of test compound/OD of control compound}] \times 100.$$

4.6. In silico pharmacological property and SAR study

The pharmacological properties of the compounds such as molecular weight, *c* Log *P* and quantum chemical descriptors such as E_{HOMO} (energy of highest occupied molecular orbital) and E_{LUMO} (energy of lowest unoccupied molecular orbital) of the synthesized compounds were calculated using a BioMed CaChe 6.1 (FujiSuit Ltd), a computer aided molecular design modeling tool for windows ME 9820000 and XP operating system. Other parameters like HBA, HBD, molecular PSA, ionization potential, drug score and drug likeness of the compounds were also studied using online Osiris property explorer for drug bioavailability of chemical compounds.[19]

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