Bioorganic & Medicinal Chemistry 17 (2009) 8168–8173

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of a novel series of pyrazole chalcones as anti-inflammatory, antioxidant and antimicrobial agents

Babasaheb P. Bandgar [a,b,*], Shrikant S. Gawande [b], Ragini G. Bodade [c], Nalini M. Gawande [d], Chandrahasya N. Khobragade [c]

[a] Organic Chemistry Research Laboratory, School of Chemical Sciences, Solapur University, Solapur 413 255, India
[b] Organic Chemistry Research Laboratory, School of Chemical Sciences, Swami Ramanand Teerth Marathawada University, Nanded 431 606, India
[c] Biochemistry Research Laboratory, School of Life Sciences, Swami Ramanand Teerth Marathawada University, Nanded 431 606, India
[d] Bilcare Clinical Research Academy, Sai Capital, Senapati Bapat Road, Pune 411 016, India

ARTICLE INFO

Article history:
Received 9 October 2009
Revised 16 October 2009
Accepted 17 October 2009
Available online 22 October 2009

Keywords:
Pyrazole chalcones
Anti-inflammatory activity
Antioxidant activity
Antimicrobial activity

ABSTRACT

A novel series of 1-(2,4-dimethoxy-phenyl)-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-propenone (**3**) have been prepared by the Claisen–Schmidt condensation of 1-(2,4-dimethoxy-phenyl)-ethanone (**1**) and substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes (**2**). Substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes (**2**) were prepared by Vilsmeir–Haack reaction on acetophenonephenylhydrazones to offer the target compounds. The structures of the compounds were established by IR, ^{1}H NMR and mass spectral analysis. All the compounds were evaluated for their anti-inflammatory (TNF-α and IL-6 inhibitory assays), antioxidant (DPPH free radical scavenging assay) and antimicrobial activities (agar diffusion method) against some pathogenic bacteria and fungi. Of 10 compounds screened, compounds **3a**, **3c** and **3g** exhibited promising IL-6 inhibitory (35–70% inhibition, 10 μM), free radical scavenging (25–35% DPPH activity) and antimicrobial activities (MIC 100 μg/mL and 250 μg/mL) at varied concentrations. The structure–activity relationship (SAR) and in silico drug relevant properties (HBD, HBA, PSA, *c* Log *P*, molecular weight, E_{HOMO} and E_{LUMO}) further confirmed that the compounds are potential lead compounds for future drug discovery study. Toxicity of the compounds was evaluated theoretically and experimentally and revealed to be nontoxic except **3d** and **3j**.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are therapeutically important in the treatment of rheumatic arthritis and in various types of inflammatory conditions, but they are found to be limited because of their frequently observed gastrointestinal side effects. Thus, there is an urgent need for new targets that are required for the design and development of novel anti-inflammatory agents as an alternative to NSAIDs.[1] Tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), the two important multifunctional proinflammatory cytokines, are involved in the pathogenesis of autoimmune, inflammatory, cardiovascular, neurodegenerative and cancer diseases through a series of cytokine signaling pathways.[2,3] IL-6 contributes to the initiation and extension of the inflammatory process, therefore it is considered as a central mediator in a range of inflammatory diseases but has not received the desired attention in drug discovery.[4] TNF-α and IL-6 are thus pharmaceutically important molecular targets for the treatment of the above-mentioned diseases. Reactive oxygen species are implicated in the induction and prolongation of inflammatory process.[5] Interestingly a number of therapeutically useful NSAID's have been shown to act by virtue of their free radical scavenging activity.[6–8] Antioxidants are the compounds that prevent oxidative damage induced by free radicals and ROS. Thus, antioxidant therapy has also gained immense importance in the treatment of the above-mentioned diseases.[9]

Flavonoids are widely distributed phytochemicals classified as anthocyanidins, flavonols, chalcones, aurones, flavanone, isoflavones, flavans, flavanonols, flavanols and flavones differencing in their structural group arrangements.[10] Chalcone, an important intermediate of flavonoid synthetic pathway exerts a great deal of biological and pharmacological activities viz. antimicrobial, anti-anginal, anti-hepatotoxic, anti-inflammatory, antioxidant, anticancer, antimalarial and anti-allergic.[11,12] On the other hand, pyrazoles are of interest as potent bioactive molecules. They are known to exhibit pharmaceutical activities such as CNS depressant, neuroleptic, tuberculostatic, antihypertensive, antileishmanial, analgesic, antidiabetic, antitumor and antimicrobial.[13,14] Moreover, pyrazole derivatives find applications as dyestuffs, analytical reagents and agrochemicals.[15]

Anti-inflammatory activity of the chalcones was confirmed in several in vivo clinical trials by the inhibition of cell migration and TNF-α synthesis, while antioxidant activity is related with its

* Corresponding author. Tel./fax: +91 217 2351300.
E-mail address: bandgar_bp@yahoo.com (B.P. Bandgar).

0968-0896/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2009.10.035

free radical scavenging ability attributed to phenolic –OH group attached to the ring structure.[16,17]

Synthesis of flavonols having pyrazole moiety at C-2 position was recently reported as potent antifungal and antibacterial agents.[18,19] Furthermore, the presence of enone function in chalcone moiety with pyrazole ring also enhanced the biological activity.[20]

Prompted by all these observations, we report here the synthesis and biological activity of pyrazole chalcones as anti-inflammatory, antioxidant and antimicrobial agents. The structure–activity relationship (SAR) was studied to explore its biological activities using pharmacological parameters such as E_{HOMO}, E_{LUMO}, *c* Log *P*, hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs), calculated using computational softwares. The toxicity of the compounds was evaluated theoretically and experimentally to determine their potential as safe leading compounds for bioavailability.

2. Results and discussion

2.1. Chemistry

In the present investigation 1-(2,4-dimethoxy-phenyl)-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-propenones (**3**) have been prepared by the Claisen–Schmidt condensation of 1-(2,4-dimethoxy-phenyl)-ethanone (**1**) and substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes (**2**) by the known literature method[21] (Scheme 1). Substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes (**2**) were prepared by Vilsmeir–Haack reaction on acetophenonephenylhydrazones.[22] The residue was purified on column chromatography (silica gel with 10% ethyl acetate in hexane). The compounds structure (Fig. 1) was confirmed by spectral data (IR, ^{1}H NMR and MS). The chemical profile of the compounds is as shown in Table 1.

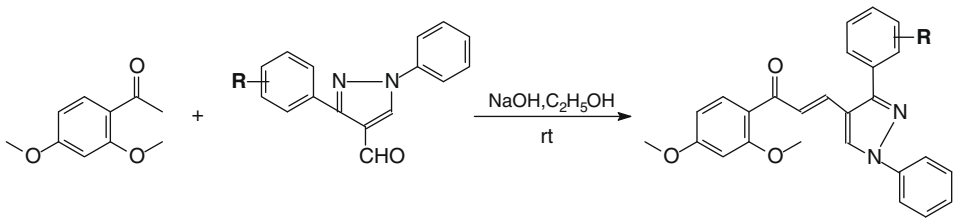
2.2. Biological evaluation

All the synthesized compounds were evaluated for anti-inflammatory activity by TNF-α and IL-6 inhibition assays. None of the compounds showed a promising TNF-α inhibitory activity except **3d** and **3e** up to 1–2% at 10 μM concentration, while a promising IL-6 inhibitory activity was shown by **3a**, **3c**, **3g**, **3i** and **3j** up to 35–70% inhibition at 10 μM concentration. As compared to the standard dexamethasone, the activity results are revealed to be comparable as summarized in Table 2. Most of the compounds did not show significant cytotoxicity at 10 μM concentration except **3d** and **3j**.

DPPH is the well-known method for determining antioxidant activity of plant flavonoids. Antioxidant activity of the compounds is summarized in Table 3. DPPH radical scavenging activity of the compounds was found to be good to moderate as compared to the standard BHA. The compounds **3a**, **3c**, **3j**, **3f**, **3d**, **3g**, **3i** and **3e** stabilize the DPPH in the range of 15–35% except **3b** and **3h**. Antioxidant activity of the compounds is related with their electron or hydrogen radical releasing ability to DPPH so that they become stable diamagnetic molecules. This might be the reason for the higher antioxidant activity of the above-mentioned compounds. Antioxidant activity of the compounds is also well explained by E_{HOMO} and E_{LUMO}, as the electron donation property has been strongly attributed to E_{HOMO} (electron donation capability) and E_{LUMO} (electron accepting capability). The compounds possessing higher E_{HOMO} and E_{LUMO} were found to be effective agents for stabilizing the DPPH radicals.

A broad spectrum antifungal activity of the compounds **3c**, **3b**, **3a**, **3j**, **3f** and **3e** was obtained against all the fungi at MIC 250 μg/mL, while other compounds revealed a slight antifungal activity. Fungi *Aspergillus flavus* was observed to be more sensitive than *Trichoderma viridae* and *Aspergillus niger*. Of the five tested bacterial strains, *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* were inhibited mostly by compounds **3c**, **3d**, **3b**, **3e**, **3i** and **3j** at MIC 100 g/mL as listed in Table 4. Regarding the SAR study from the analysis data, it is suggested that compounds substituted with electron-releasing groups (OCH_3 > CH_3 > F > Br > Cl) increase the antimicrobial activity. It is also reported that a part from the active moiety (1-(2,4-dimethoxy-phenyl)-propenone), substituent position in the phenyl ring is also found to be biologically relevant. Our antimicrobial activity data confirmed that the substitution of Cl at *m* position is more relevant for increasing its biological activity than at *o* and *p* positions. This is also supported by the previous report.[23,24]

To qualify the compound as a drug candidate, it is analyzed by the parameters set by Lipinski's rule of five using Osiris property explorer. The *c* Log *P* is the important physiochemical property indicating the lipophilicity and the ability of molecule to cross the various biological membranes. According to Lipinski's rule of five the *c* Log *P* value below 5 is feasible for a compound to be a future drug. The synthesized compounds showed a marginal lipophilicity within the range of 4.0–5.0. The molecular weight property of the compound is related to its in vivo administration. All the synthesized compounds have the molecular weight within the acceptable range, that is, 400–500. The compounds showed the HBA below 10 and HBD below 5, which is also within the limit set by Lipinski's rule. The polar surface area (PSA) > 140 A^2 is thought to have low oral bioavailability, which is also revealed within the range for these compounds. Interestingly the compounds also presented a better drug likeness values. Overall, the compounds **3a**, **3c** and **3g** showed a good drug score, calculated by combining all parameters. Drug toxicity is a factor of great importance for a potential commercial drug, since a significant number of drugs are disapproved in clinical trials based on their high toxicity profile. The toxicity of the compounds is calculated in terms of mutagenicity, tumorigenic, reproductively effective and irritant. All the compounds were confirmed as non-mutagenic and therefore were



1-(2,4-Dimethoxy-phenyl)-ethanone — **1**

1,3-Diphenyl-1*H*-pyrazole-4-carbaldehyde — **2**

1-(2,4-Dimethoxy-phenyl)-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-propenone — **3a-j**

Scheme 1.

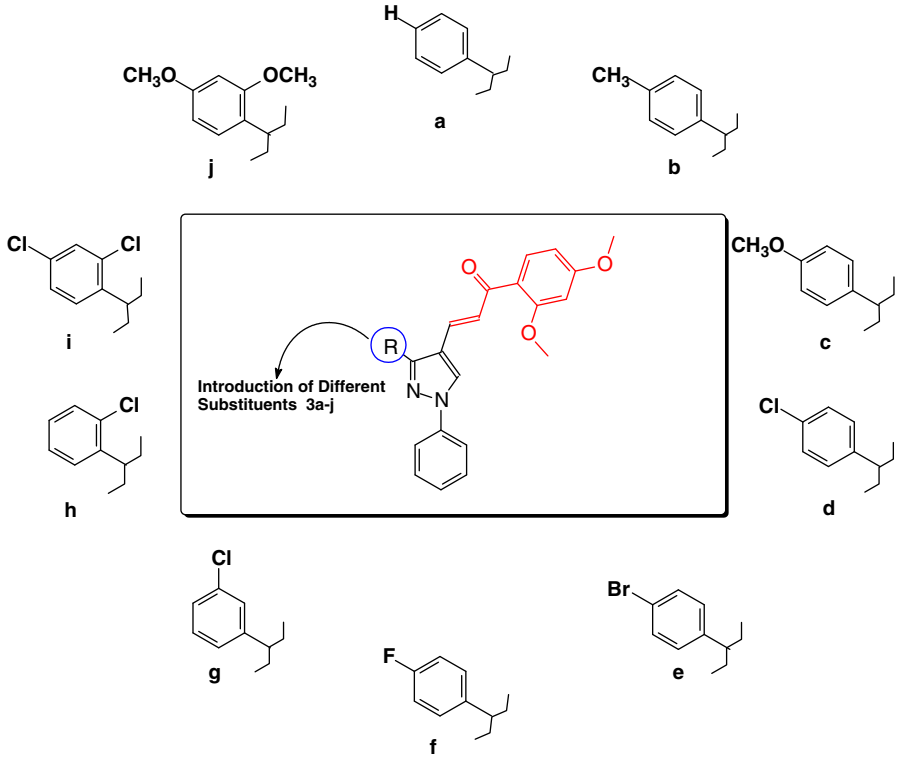


Figure 1. Structure of pyrazole chalcones.

Table 1
Characterization of compounds and in silico pharmacological parameters for bioavailability

Entry	Product (**3**)	Molecular formula	Molecular weight	Yield (%)	mp (°C)	E_{HOMO}	E_{LUMO}	HBD	HBA	Mol. PSA	*c* Log *P*	Solubility	Drug likeness	Drug score
3a	R = 4-H	$C_{26}H_{20}Cl_2N_2O_3$	410	67	157	−8.6	−0.745	0	4	44.22	4.16	−5.39	−0.3	0.37
3b	R = 4-CH_3	$C_{27}H_{24}N_2O_3$	424	72	168	−8.59	−0.728	0	4	44.22	4.48	−5.73	−1.56	0.27
3c	R = 4-OCH_3	$C_{27}H_{24}N_2O_4$	440	70	120	−8.43	−0.731	0	5	53.08	4.06	−5.41	0.05	0.38
3d	R = 4-Cl	$c_{26}h_{21}n_2o_3$	444	84	149	−8.62	−0.802	0	4	44.22	4.78	−6.13	0.76	0.34
3e	R = 4-Br	$C_{26}H_{21}BrN_2O_3$	488	58	171	−8.78	−0.824	0	4	44.22	4.86	−6.22	−2.11	0.2
3f	R = 4-F	$C_{26}H_{21}FN_2O_3$	428	62	132	−8.78	−0.826	0	4	44.22	4.22	−5.7	−0.99	0.3
3g	R = 3-Cl	$C_{26}H_{21}CW_2O_3$	444	72	127	−8.68	−0.793	0	4	44.22	4.78	−6.13	−0.31	0.29
3h	R = 2-Cl	$C_{26}H_{21}ClN_2O_3$	444	70	121	−8.84	−0.745	0	4	44.22	4.78	−6.13	0.32	0.32
3i	R = 2,4-Cl	$C_{26}H_{20}CL_2N_2O_3$	478	68	162	−8.89	−0.796	0	4	44.22	5.39	−6.86	0.93	0.27
3j	R = 2,4-OCH_3	$C_{28}H_{26}N_2O_5$	470	64	110	−8.65	−0.65	0	6	61.05	3.95	−5.43	−0.4	0.34

Table 2
Anti-inflammatory activity against TNF-α and IL-6

Entry	% Inhibition at 10 μM		
	TNF-α	IL-6	Toxicity
3a	0	64.72	0
3b	0	0	0
3c	0	38.16	0
3d	1.13	2.71	11.488
3e	1.81	11.3	0
3f	0	0	0
3g	0	67.48	0
3h	0	14.5	0
3i	0	40.78	0
3j	0	37.94	2.5107
Dexamethasone (1 μM/mL)	69	90	0

biologically safe for intake. The toxicity of the compounds was thus confirmed theoretically and experimentally.

3. Conclusion

The synthesized novel series of pyrazole chalcones were studied for their in vitro biological activity. The compounds were also tested for their bioavailability by in silico study. SAR study confirmed that **3a**, **3c** and **3g** are potent lead compounds for drug discovery with negligible toxicity.

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 a Bruker Avance (400 MHz) spectrometer in $CDCl_3$ solutions, with TMS as an internal reference. Mass spectra were recorded on a Shimadzu GCMS-

Table 3
% DPPH activity

Entry	% DPPH[b]
3a	31.79 ± 0.22
3b	13.5 ± 0.21
3c	34.64 ± 0.18
3d	18.68 ± 0.16
3e	17.24 ± 0.22
3f	26.68 ± 0.25
3g	29.68 ± 0.19
3h	13.81 ± 0.23
3i	18.25 ± 0.15
3j	30.33 ± 0.20
BHA[a]	66.78 ± 0.21

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

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 pyrazole chalcones (3a–j)

A mixture of 1-(2,4-dimethoxy-phenyl)-ethanone **1** (0.180 g, 1 mmol) and 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde **2** (0.248 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 2.5 h. Then this reaction mixture was poured over crushed ice and acidified with dil HCl. The 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 1-(2,4-dimethoxy-phenyl)-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-propenone (**3a**) (Scheme 1).

The physical and spectral data of new 1-(2,4-dimethoxy-phenyl)-ethanone chalcone analogues of pyrazoles (**3a–j**) are given below.

4.2.1. 1-(2,4-Dimethoxy-phenyl)-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-propenone (3a)

Light yellow solid, mp: 157 °C, IR (KBr): ν_{max}: 3025, 2941, 1668, 1601, 1411, 1234, 825 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.26 (s, 1H), 7.79 (d, 1H, $J = 7.5$ Hz), 7.75 (m, 2H), 7.57 (d, 2H, $J = 15.7$ Hz), 7.46 (m, 3H), 7.32 (m, 3H), 7.31 (d, 1H, $J = 15.5$ Hz), 6.56 (dd, 1H, $J = 2$ Hz, 2 Hz), 6.47 (d, 1H, $J = 2$ Hz), 3.86 (s, 3H), 3.84 (s, 3H); $m/z = 411$ (M+1).

4.2.2. 1-(2,4-Dimethoxy-phenyl)-3-(1-phenyl-3-p-tolyl-1*H*-pyrazol-4-yl)-propenone (3b)

Light yellow solid, mp: 168 °C, IR (KBr): ν_{max}: 3027, 2944, 1670, 1604, 1414, 1231, 822 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$):δ 8.24 (s, 1H), 7.78 (d, 1H, $J = 7.6$ Hz), 7.74 (m, 2H), 7.62 (d, 2H, $J = 15.6$ Hz), 7.45 (m, 2H), 7.31 (m, 2H), 7.21 (d, 1H, $J = 15.6$ Hz), 6.55 (dd, 1H, $J = 2$ Hz, 2 Hz), 6.46 (d, 1H, $J = 2$ Hz), 3.98 (s, 3H), 3.84 (s, 3H), 2.46 (s, 3H); $m/z = 425$ (M+1).

4.2.3. 1-(2,4-Dimethoxy-phenyl)-3-[3-(4-methoxy-phenyl)-1-(1-methyl-buta-1,3-dienyl) -1*H*-pyrazol-4-yl]-propenone (3c)

Yellow solid, mp: 120 °C, IR (KBr): ν_{max}: 3092, 2930, 1675, 1617, 1420, 1235, 835 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.24 (s, 1H), 7.77 (d, 1H, $J = 7.6$ Hz), 7.72 (m, 2H), 7.64 (d, 1H, $J = 15.6$ Hz), 7.48 (m, 3H), 7.30 (m, 2H), 7.00 (d, 1H, $J = 15.6$ Hz), 6.56 (dd, 1H, $J = 2$ Hz, 2 Hz), 6.47 (d, 1H, $J = 2$ Hz), 3.86 (s, 3H), 3.88 (s, 3H), 3.84 (s, 3H) ; $m/z = 440$ (M^+).

4.2.4. 3-[3-(4-Chloro-phenyl)-1-phenyl-1*H*-pyrazol-4-yl]-1-(2,4-dimethoxy-phenyl)-propenone (3d)

Yellow solid, mp: 149 °C, IR (KBr): ν_{max}: 3056, 2937, 1673, 1605, 1425, 1209, 826 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.23 (s, 1H), 7.79–7.75 (m, 4H), 7.66 (d, 1H, $J = 1$ 5.9 Hz), 7.50 (m, 2H), 7.48 (m, 1H), 7.45 (d, 1H, $J = 15.9$ Hz), 7.35 (m, 3H), 6.57 (dd, 1H, $J = 2$ Hz, 2 Hz), 6.48 (d, 1H, $J = 2$ Hz), 3.87 (s, 3H), 3.85 (s, 3H) ; $m/z = 445$ (M^+).

4.2.5. 3-[3-(4-Bromo-phenyl)-1-phenyl-1*H*-pyrazol-4-yl]-1-(2,4-dimethoxy-phenyl)-propenone (3e)

Light yellow solid, mp: 171 °C, IR (KBr): ν_{max}: 3059, 2939, 1676, 1609, 1427, 1211, 829 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.19 (s, 1H), 7.79–7.77 (m, 4H), 7.62 (d, 1H, $J = 15.8$ Hz), 7.59 (m, 2H), 7.46–7.41 (m, 1H), 7.31 (d, 1H, $J = 15.9$ Hz), 7.29–7.25 (m, 3H), 6.58 (dd, 1H, $J = 2$ Hz, 2 Hz), 6.41 (d, 1H, $J = 2$ Hz), 3.84 (s, 3H), 3.83 (s, 3H); $m/z = 490$ (M^+).

4.2.6. 1-(2,4-Dimethoxy-phenyl)-3-[3-(4-fluoro-phenyl)-1-phenyl-1*H*-pyrazol-4-yl]-propenone (3f)

Light yellow solid, mp: 132 °C, IR (KBr): ν_{max}: 3058, 2940, 1676, 1611, 1426, 1211, 829 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.24 (s, 1H), 7.79–7.76 (m, 4H), 7.69 (d, 1H, $J = 16.1$ Hz), 7.51–7.49 (m,

Table 4
Antimicrobial activity of pyrazole chalcone derivatives (zone of inhibition in mm)

Compound	Bacteria (MIC at 100 μg/mL)						Fungi (MIC at 250 μg/mL)	
	EC	KP	PV	SA	BS	AN	TV	AF
3a	10	—	—	8	10	12	14	15
3b	11	12	—	9	8	14	15	12
3c	8	±	7	11	10	15	16	14
3d	11	—	12	11	13	11	13	10
3e	9	10	—	8	7	11	13	13
3f	12	—	10	15	14	13	14	12
3g	9	±	8	6	8	9	11	10
3h	12	—	—	8	7	11	12	9
3i	11	—	—	10	9	8	10	10
3j	10	8	—	7	6	12	14	13
Tetracycline	—	32	20	17	—		—	—
Nystatin	—	—	—	—	—	11	12	11
Control	—	±	—	±	—	±	±	±

Data represent the 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); not detected, — ; trace activity, ±.

3H), 7.37–7.35 (m, 3H), 7.10 (d, 1H, J = 16.1 Hz), 6.59 (dd, 1H, J = 2 Hz, 2 Hz), 6.50 (d, 1H, J = 2 Hz), 3.84 (s, 3H), 3.82 (s, 3H) ; m/z = 429 (M+1).

4.2.7. 3-[3-(3-Chloro-phenyl)-1-phenyl-1*H*-pyrazol-4-yl]-1-(2,4-dimethoxy-phenyl)-propenone (3g)

Light yellow solid, mp: 127 °C, IR (KBr): ν_{max}: 3060, 2941, 1674, 1608, 1430, 1212, 825 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.19 (s, 1H), 7.77–7.75 (m, 4H), 7.65 (d, 1H, J = 16.4 Hz), 7.59–7.57 (m, 2H), 7.45–7.40 (m, 1H), 7.35 (m, 3H), 7.2 (d, 1H, J = 16.4 Hz), 6.49 (dd, 1H, J = 2 Hz, 2 Hz), 6.40 (d, 1H, J = 2 Hz), 3.81 (s, 3H), 3.80 (s, 3H) ; m/z = 445 (M^+), 447 (M+2).

4.2.8. 3-[3-(2-Chloro-phenyl)-1-phenyl-1*H*-pyrazol-4-yl]-1-(2,4-dimethoxy-phenyl)-propenone (3h)

Light yellow solid, mp: 121 °C, IR (KBr): ν_{max}: 3062, 2943, 1679, 1610, 1431, 1214, 832 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.43 (s, 1H), 7.79–7.77 (m, 4H), 7.65 (d, 1H, J = 16.4 Hz), 7.58–7.56 (m, 3H), 7.41–7.39 (m, 1H), 7.35 (m, 3H), 7.1 (d, 1H, J = 15.7 Hz), 6.43 (dd, 1H, J = 2 Hz, 2 Hz), 6.39(d, 1H, J = 2 Hz), 3.85 (s, 3H), 3.84 (s, 3H) ; 445 (M^+).

4.2.9. 3-[3-(2,4-Dichloro-phenyl)-1-phenyl-1*H*-pyrazol-4-yl]-1-(2,4-dimethoxy-phenyl)-propenone (3i)

Yellow solid, mp: 162 °C, IR (KBr): ν_{max}: 3061, 2957, 1680, 1632, 1427, 1212, 830 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.22 (s, 1H), 7.79–7.76 (m, 3H), 7.59 (d, 1H, J = 16.2 Hz), 7.58–7.57 (m, 2H), 7.44–7.41 (m, 1H), 7.35 (m, 3H), 7.19 (d, 1H, J = 16.0 Hz), 6.59 (dd, 1H, J = 2 Hz, 2 Hz), 6.44 (d, 1H, J = 2 Hz), 3.83 (s, 3H), 3.81 (s, 3H) ; m/z = 479 (M^+).

4.2.10. 1-(2,4-Dimethoxy-phenyl)-3-[3-(2,4-dimethoxy-phenyl)-1-phenyl-1*H*-pyrazol-4-yl]-propenone (3j)

Yellow solid, mp: 110 °C, IR (KBr): ν_{max}: *3096*, 2935, 1680, 1619, 1428, 1240, 838 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.22 (s, 1H), 7.76 (d, 1H), 7.75–7.73 (m, 3H), 7.64 (d, 1H, J = 16.2 Hz), 7.48–7.47 (m, 3H), 7.30–7.28 (m, 2H), 7.21 (d, 1H, J = 16.2 Hz), 6.59 (dd, 1H, J = 2 Hz, 2 Hz), 6.45 (d, 1H, J = 2 Hz), 3.86 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H) ; m/z = 471 (M+1).

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. 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.[25] The 50% inhibitory concentration (IC_{50}) values were calculated by a nonlinear regression method as shown in Table 2.

4.4. In vitro antioxidant activity (DPPH method)

The compounds (**3a**–**j**) were evaluated for their in vitro free radical scavenging activity by the 2,2′diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method.[26]

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. The data are summarized in Table 3.

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

4.5. Antimicrobial activity (agar diffusion method)

Antimicrobial activity of all synthesized compounds was determined by agar diffusion method.[27,28] All human pathogenic bacteria viz. *Bacillus megaterium* (MTCC 1684), *Bacillus subtilis* (MTCC 1789), *Klebsiella pneumoniae* (NCIM 2957), *Staphylococcus aureus* (MTCC 96), *Proteus vulgaris* (MTCC 1771), *Escherichia coli* (MTCC 1650) and fungi viz. *Trichoderma viridae* (MTCC 167), *Aspergillus flavus* (MTCC 2501) and *Aspergillus niger* (MTCC 1781) were procured from the Institute of Microbial Technology (IMTech), Chandigarh, India and the National Collection of Industrial Microorganisms (NCIM), Pune, India. Stock solutions of compounds were diluted in dimethyl sulfoxide (1% DMSO) to give a final concentration ranging from 50 to 500 μg/mL for determining the MIC value. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of compound required for a 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 spread on Sabouraud's Dextrose agar plates (HiMedia). For antibacterial activity Muller Hinton agar was used (HiMedia) seeded with 0.1 mL of the respective bacterial culture strains suspension prepared in a 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 a control. The antibiotic nystatin (30 μg/mL) and tetracycline (10 μg/mL) are used as reference antifungal and antibacterial agents, respectively, for comparison. Inoculated plates in duplicate were then incubated at 37 ± 0.5 °C for antibacterial activity for 24 h and 48 h at 28 ± 0.2 °C for antifungal activity. After incubation the antimicrobial activity was measured in terms of the zone of inhibition in mm as shown in Table 4.

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 such as HBA, HBD, molecular PSA, drug score and drug likeness of the compounds were also studied using online Osiris property explorer for drug bioavailability of chemical compounds. Since compounds are considered for oral delivery, they were also assessed for toxicity using in silico ADME prediction methods.[23,24]

Acknowledgements

The authors are thankful to Dr. Somesh Sharma, Piramal Life Sciences, Mumbai, for anti-inflammatory activity and to the

Director, School of Life Sciences, Swami Ramanand Teerth Marathawada University, Nanded, for antioxidant and antimicrobial activities.

References and notes

1. Shishoo, C. J.; Ravikumar, T.; Jain, K. S.; Rathod, I. S.; Gandhi, T. P.; Satia, M. C. *Ind. J. Chem.* **1999**, *38B*, 1075.
2. Papadakis, K. A.; Targan, S. R. *Inflamm. Bowel. Dis.* **2000**, *6*, 303.
3. Krishnamoorthy, S.; Honn, K. V. *Cancer Metast. Rev.* **2006**, *25*, 481.
4. Dominic, S. C. R. Semin. Arthritis Rhumat. in press. doi: 10.1016/j.semarthrit.2008.01.006).
5. Freeman, B. A.; Crop, J. P. *Lab. Invest.* **1982**, *47*, 412.
6. Vane, J. R. *Agents React.* **1978**, *6*, 430.
7. Halliwel, B.; Gutteridge, J. M. Free Radicals Ageing and Disease. In *Free radicals in Biology and Medicine*; Halliwell, B., Gutteridge, J. M. C., Eds., 2nd ed.; Clarendon Press: Oxford, 1989; p 427.
8. Roberfroid, M. B.; Vieche, H. G.; Ramacle, J.. In *Advances in Drug Research Free radicals in Drug Research*; Academic Press, 1987; Vol. 16. Chapter 1.
9. Velavan, S.; Naghlendran, K.; Mahesh, R.; Hazeena Begum, V. *PHCOGMAG*, **1998**, ISSN: 0973-1296, 26.
10. Narayana, K.; Reddy, R. M. S.; Chaluvadi, M. R.; Krishna, D. R. *Ind. J. Pharm.* **2001**, *33*, 2.
11. Yuh-meei, L.; Yasheen, Z.; Michael, T. F.; Li-Ming, Z.; Weiguo, N.; Faching, C. *Bioorg. Med. Chem.* **2002**, *10*, 2795.
12. Nowakowska, Z. *Eur. J. Med. Chem.* **2007**, *42*, 125.
13. Holla, B. S.; Mahalinga, M.; Karthikeyan, M. S.; Akberali, P. M.; Shetty, N. S. *Bioorg. Med. Chem.* **2006**, *14*, 2040.
14. Manna, F.; Chimenti, F.; Fioravati, R.; Bolasco, A.; Seecci, D.; Chimenti, P.; Ferlini, C.; Scambia, G. *J. Bioorg. Med. Chem. Lett.* **2005**, *15*, 4632.
15. Mulder, R.; Wellinga, K.; Van Daalen, J. J. *Naturwissenschaften* **1975**, *62*, 531.
16. Herencia, F.; Ferrandiz, M. L.; Ubeda, A.; Guillen, I.; Chan, G. M. *Free Radical Biol. Med.* **2001**, *30*, 43.
17. Okawa, M.; Kinjo, J.; Nohara, T.; Ono, M. *Biol. Pharm. Bull.* **2001**, *24*, 1202.
18. Prakash, O.; Kumar, R.; Sehrawat, R. *Eur. J. Med. Chem.* **2009**, *44*, 1763.
19. Prakash, O.; Kumar, R.; Sehrawat, R. *Eur. J. Med. Chem.* **2008**, *43*, 435.
20. Joshi, N. S.; Shaikh, A. A.; Deshpande, A. P.; Karale, B. K.; Bhirud, S. B.; Gill, C. H. *Ind. J. Chem.* **2005**, *44B*, 422.
21. Jung, S. H.; Park, S. Y.; Kim-PAK, Y.; Lim, S. S. *Chem. Pharm. Bull.* **2006**, *54*, 368.
22. Kira, M. A.; Abdel-Raeman, M. O.; Gadalla, K. Z. *Tetrahedron Lett.* **1969**, *2*, 109.
23. Romeiro, N. C.; Leite, R. D. F.; Lima, L. M.; Cardozo, S. V. S.; De Miranda, A. L. P.; Fraga, C. A. M.; Barreiro, E. J. *Eur. J. Med. Chem.* **2009**, *44*, 1959.
24. Leal, B.; Afonso, I. F.; Rodrigues, C. R.; Abreu, P. A., et al *Bioorg. Med. Chem.* **2008**, *16*, 8196.
25. Hwang, C.; Catanaga, M.; Gale, A.; Gatanaga, T. *J. Immunol.* **1993**, *151*, 5631.
26. George, S.; Parameswaran, M.; Chakraborty, A. R.; Ravi, T. K. *Acta. Pharm.* **2008**, *58*, 119.
27. Fairbrother, R. W.; Martyn, G. *J. Clin. PathoL.* **1951**, *4*, 374.
28. Gould, J. C.; Bowie, J. H. *J. Edinb. Med.* **1952**, *59*, 178.