

ORIGINAL ARTICLE

## Synthesis, characterization and biological evaluation of novel 4'-fluoro-2'-hydroxy-chalcone derivatives as antioxidant, anti-inflammatory and analgesic agents

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### Abstract

In an effort to develop safe and potent anti-inflammatory agents, a series of novel 4'-fluoro-2'-hydroxychalcones **5a–d** and their dihydropyrazole derivatives **6a–d** was prepared. It was synthesized *via* aldol condensation of 4'-fluoro-2'-hydroxyacetophenone with appropriately substituted aldehydes followed by cyclization with hydrazine hydrate. All the synthesized compounds were evaluated for their antioxidant, anti-inflammatory, cyclooxygenase inhibition selectivity and analgesic activities. The dimethoxychalcone **5a** and its dihydropyrazole derivative **6a** showed the highest antioxidant activity, while the monomethoxychalcone **5d** and its dihydropyrazole derivative **6d** showed the highest analgesic and anti-inflammatory activities. It was also found that there is a close correlation between 4'-fluoro-2'-hydroxychalcones **5a–d** and their dihydropyrazole derivatives **6a–d** in the screened biological activities. To explain the correlation between the synthesized chalcones and their dihydropyrazole derivatives, especially for the anti-inflammatory activity, docking studies were performed.

### Introduction

Chalcones are precursors of flavonoid compounds, which are present in many edible plants and display interesting biological activities including antioxidant<sup>1</sup>, antibacterial<sup>2</sup>, anti-inflammatory<sup>3–5</sup> and cytotoxic<sup>4–6</sup> activities. Chemically, chalcones consist of two aromatic rings joined by a three carbon  $\alpha$ ,  $\beta$ -unsaturated carbonyl system (structure, Figure 1). It was reported that 2-hydroxyl substitution on A ring is critical for both antioxidant<sup>7</sup> and anti-inflammatory<sup>8</sup> activities of chalcones. In addition, Padhye et al.<sup>9</sup> observed that fluorination of chalcones enhances the biological activities as shown in the reported fluorinated 2'-hydroxychalcones (**1**). In addition, promising anti-inflammatory and antioxidant activities of 2,4-dimethoxy and 3,4,5-trimethoxy chalcones (**2**) were obtained<sup>10</sup>. Cyclization of the chalcones with hydrazine hydrate will yield the corresponding dihydropyrazole derivatives, which retain the geometry of the two aromatic rings separated with three bonds like chalcones. It is expected that these dihydropyrazole derivatives will maintain the same biological activities of the corresponding open chalcones. Based on these observations, we now describe the synthesis and antioxidant, analgesic and anti-inflammatory activities for a new group of 4'-fluoro-2'-hydroxychalcones **5a–d** and their cyclized

### Keywords

Analgesic, anti-inflammatory, antioxidant, cyclooxygenase inhibition, dihydropyrazole, 4'-fluoro-2'-hydroxychalcones

### History

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3,5-diaryl-4,5-dihydro-1*H*-pyrazoles **6a–d** in which (i) hydroxyl group at 2-position of A ring, (ii) fluoro substituent at 4-position of the same ring, (iii) methoxy, thiomethoxy or two methoxy groups at different positions of B ring.

### Results and discussion

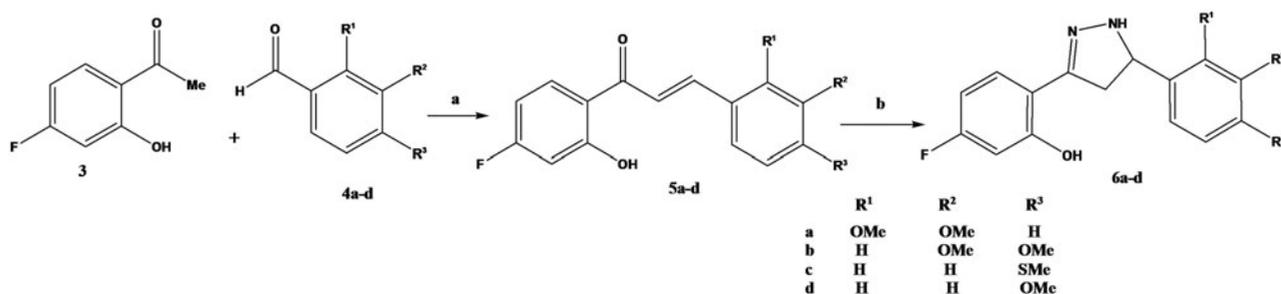
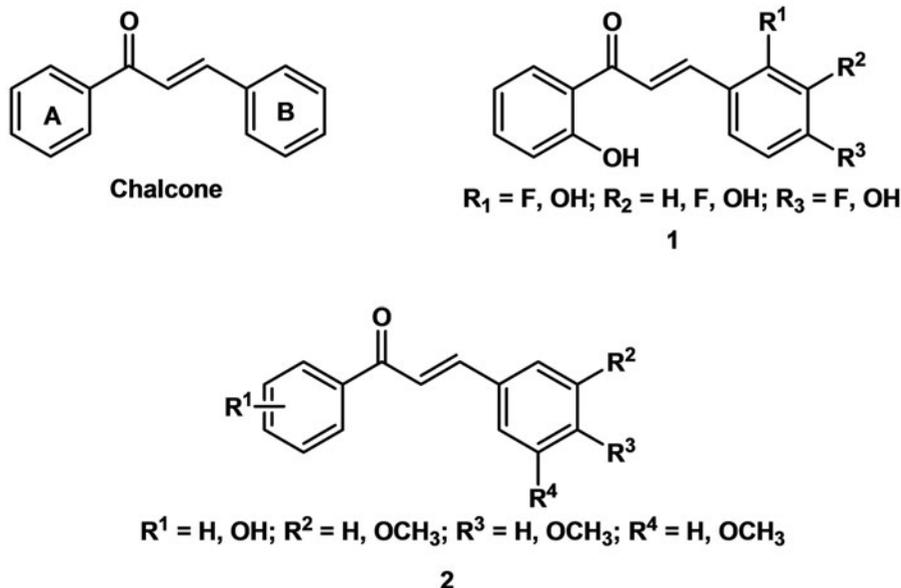
#### Chemistry

A group of 3-(4-fluoro-2-hydroxyphenyl)-5-(substitutedphenyl)-4,5-dihydro-1*H*-pyrazoles (**6a–d**) was synthesized using the reaction sequence illustrated in Scheme 1. Accordingly, reaction of 4'-fluoro-2'-hydroxyacetophenone (**3**) with different substituted benzaldehydes **4a–d** under reflux in ethanol provided the corresponding 4'-fluoro-2'-hydroxychalcones (**5a–d**) in higher yields (74–91%). Cyclization of the chalcones **5a–d** with hydrazine hydrate in ethanol gave the respective 3,5-diaryl-4,5-dihydropyrazoles (**6a–d**) in good yields (65–78%). All the prepared compounds have been characterized by infrared (IR), <sup>1</sup>H NMR, <sup>13</sup>C NMR, mass spectra and elemental analyses.

The IR spectra of chalcone compounds **5a–d** showed sharp peak at 1632–1641 cm<sup>-1</sup> corresponding to C=O, while the <sup>1</sup>H NMR spectra of **5a–d** displayed two doublets, each of one proton intensity, one at  $\delta$  7.43–7.68 and the second at  $\delta$  7.90–8.21 with high *J* value (15.6 Hz) indicating the two olefinic protons. The <sup>1</sup>H NMR spectra of dihydropyrazole compounds **6a–d** showed three signals as doublet of doublet (dd), each of one proton intensity, one at  $\delta$  3.09–3.30, second at  $\delta$  3.56–3.67 and the third at  $\delta$  4.97–5.23 with three different *J* values (16.5, 10.2 and 8.7 Hz,

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Figure 1. Chemical structures of chalcone, fluorinated 2'-hydroxychalcones (1) and methoxylated chalcones (2).



Scheme 1. Reagents and conditions: (a) EtOH, NaOH, RT and 2–6 h; (b) EtOH, hydrazine hydrate, reflux and 2–4 h.

respectively) corresponding to three protons of the dihydropyrazole ring. The highest  $J$  value was due to geminal coupling of two protons at position four, while the other two  $J$  values due to coupling of two geminal protons with proton at position five. In addition,  $^{13}\text{C}$  NMR spectra of **5a–d** confirmed the presence of  $\alpha,\beta$ -unsaturated carbonyl system of chalcones by presence of three peaks at  $\delta$  121.3–125.0, 140.7–145.9 and 192.4–192.5 corresponding to propenone C-2, propenone C-1 and C of C=O, respectively. Furthermore,  $^{13}\text{C}$  NMR spectra of **6a–d** confirmed the presence of the dihydropyrazole ring due to the presence of three peaks at  $\delta$  40.3–40.9, 60.3–60.9 and 155.7–159.6 corresponding to dihydropyrazole C-4, C-5 and C-3, respectively.

## Biological evaluation

### Antioxidant activity

The antioxidant activities of the all prepared chalcones **5a–d** and their cyclized dihydropyrazoles **6a–d** were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and yeast-based antioxidant screening assay. The dimethoxychalcone **5a** was found to be the most potent antioxidant among all the chalcones **5a–d** with the least value of  $\text{IC}_{50}$  (190.56  $\mu\text{g}/\text{mL}$ ) (Table 1) and the least active among the series being compound **5c** with the largest value of  $\text{IC}_{50}$  (283.13  $\mu\text{g}/\text{mL}$ ). Similarly, for dihydropyrazole series **6a–d**, the dimethoxy derivative **6a** was found to be the most potent antioxidant among all the series with the least value of  $\text{IC}_{50}$  (79.80  $\mu\text{g}/\text{mL}$ ) and the least active among the series being compound **6c** with the largest value of  $\text{IC}_{50}$  (199.2  $\mu\text{g}/\text{mL}$ ). Furthermore, the antioxidant activity of test compounds **5a–d** and **6a–d**, based on their ability to inhibit the

Table 1. Free radical scavenging capacities of the test compounds **5a–d**, **6a–d** measured by DPPH assay.

Compounds	Mean $\text{IC}_{50} \pm \text{SD}^*$ ( $\mu\text{g}/\text{mL}$ )
<b>5a</b>	190.56 $\pm$ 8.51
<b>5b</b>	225.07 $\pm$ 8.89
<b>5c</b>	283.13 $\pm$ 16.03
<b>5d</b>	263.77 $\pm$ 12.70
<b>6a</b>	79.80 $\pm$ 15.48
<b>6b</b>	101.17 $\pm$ 9.70
<b>6c</b>	199.20 $\pm$ 26.51
<b>6d</b>	160.50 $\pm$ 9.64

\*Values of  $\text{IC}_{50}$  are expressed as means  $\pm$  SD ( $n = 3$ ).

$\text{H}_2\text{O}_2$ -induced yeast oxidative stress, showed the same order of potency (**5a** and **6a** are the most active derivatives for **5a–d** and **6a–d**, respectively, while **5c** and **6c** are the least active derivatives) as shown in Figure 2.

### Anti-inflammatory activity

*In vitro* cyclooxygenase (COX) assay revealed the structure–activity relationships of chalcones **5a–d** and the corresponding dihydropyrazoles **6a–d** and its broad range (moderately potent-to-inactive; Table 2) of inhibitory activity for COX-1 ( $\text{IC}_{50} = 14.68$  to  $> 30 \mu\text{M}$ ) and COX-2 ( $\text{IC}_{50} = 0.93$ – $12.35 \mu\text{M}$ ). Within the chalcone series **5a–d**, compound **5a** was more potent inhibitor of COX-1 than **5d**, while **5b** and **5c** were inactive. On the other hand,

all compounds inhibited COX-2 in the following order **5d** > **5b** > **5c** > **5a**. For dihydropyrazoles **6a-d**, the inhibition of COX-1 was in the following order, **6a** > **6b** > **6d**, while **6c** was inactive. Similarly, all compounds **6a-d** inhibited COX-2. These results indicated that compounds having R<sup>1</sup> = R<sup>2</sup> = H and R<sup>3</sup> = OMe (**5d** and **6d**) exhibited the highest COX-2 inhibitory activity and selectivity index. For example, **5d** had COX-2 IC<sub>50</sub> = 1.53 μM and COX-2 S.I. = 12.58, while the most potent one (**6d**) showed COX-2 IC<sub>50</sub> = 0.93 μM and COX-2 S.I. = 18.79 in comparison with the reference drug celecoxib (COX-2 IC<sub>50</sub> = 0.43 μM, S.I. = 35.51).

In addition, the anti-inflammatory activity of all the prepared chalcones **5a-d** and their cyclized dihydropyrazoles **6a-d** were evaluated using carrageenan-induced rat paw edema assay. Each compound was administered orally (10 mg/kg) immediately prior to induction of inflammation by carrageenan subcutaneous injection. The anti-inflammatory activity was then calculated based on paw-volume changes at 1, 3 and 6 h after carrageenan injection as listed in Table 2.

A comparable study of the anti-inflammatory activity of the test compounds relative to celecoxib as a reference drug at different time intervals indicated that after 1 h, the chalcone series **5a-d** showed percentage activity 24–62% and compound **5d** > **5b** > **5c** > **5a** in potency, while the dihydropyrazole series **6a-d** showed percentage activity 6–64% and compound **6d** > **6b** > **6c**

> **6a** in potency. After 3 h, the chalcone series **5a-d** showed percentage activity 32–62% and compound **5d** > **5a** > **5c** > **5b** in potency, while the dihydropyrazole series **6a-d** showed percentage activity 10–73% and compound **6d** = **6b** > **6a** > **6c** in potency. After 6 h, the chalcone series **5a-d** showed percentage activity 19–54% and compound **5d** > **5c** > **5b** > **5a** in potency, while the dihydropyrazole series **6a-d** showed percentage activity 6–74% and compound **6d** > **6b** > **6c** > **6a** in potency. 1-(4-Fluoro-2-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**5d**) displayed good anti-inflammatory activity (54–62%) comparable to that of celecoxib (32–78%) and was the most potent among the four compounds of chalcone series **5a-d** at all-time intervals. Furthermore, the corresponding cyclized dihydropyrazole derivative **6d** showed excellent anti-inflammatory activity (64–74%) and was the most potent among all compounds of dihydropyrazole series **6a-d** at all-time intervals. Similarly, dimethoxychalcone **5b** showed considerable activity (30–38%) at the three different time intervals, and its cyclized derivative **6b** displayed excellent activity (62–73%) at the same time intervals. These data indicate that (i) presence of methoxy group at 4-position of B ring of chalcones is important for anti-inflammatory activity and (ii) cyclization of chalcones to the corresponding dihydropyrazole analogues maintain or increase the anti-inflammatory activity.

#### Analgesic activity

Next, the analgesic activity of test compounds was also investigated. It was assessed by two different models: the hot-plate test and acetic acid-induced writhing test. In the hot-plate assay, the latency time on the hotplate was significantly delayed after the administration of tested compounds **5d**, **6b**, **6c** and **6d**, compared with basal values, indicating decreased nociception. The percentage increase in the pain threshold for these compounds were 35.2 and 3.52% for compound **5d** at 1 and 2 h post-treatment, 30.3 and 20.0% for compound **6b** at 1 and 2 h post-drug, 33.5% and 33.7% for compound **6c** at 1 and 2 h post-treatment and 68.5% and 50.4% for compound **6d** at 1 and 2 h post-treatment. All other compounds showed weak analgesic activity that did not reach statistical significance (Table 3).

Similarly, acetic acid-induced writhing was significantly reduced in mice receiving the compounds **5d**, **6b** and **6d**. The degree of inhibition of the writhing response by these compounds was 27.6%, 31.0% and 54.6%, compared to the vehicle-treated control group. The analgesic activity of the compound **6d** was comparable to that of the control drug, piroxicam (62.9%). Other compounds did not exhibit analgesic activity in this test either (Figure 3). From these results, the analgesic activities of these

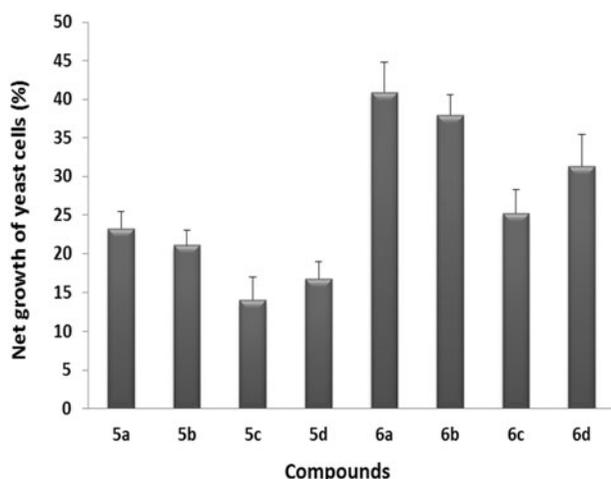


Figure 2. Antioxidant activity screening in a 96-well microplate high throughput assay using *Saccharomyces cerevisiae*. Yeast oxidative stress was measured on the basis of survival of yeast cells (yeast growth) after treatment with H<sub>2</sub>O<sub>2</sub>.

Table 2. *In vitro* COX-1 and COX-2 inhibition and anti-inflammatory activity of 4'-fluoro-2'-hydroxychalcones **5a-d**, dihydropyrazoles **6a-d** and celecoxib.

Treatment	Paw volume change (ml)* (% change)			COX Inhibition (IC <sub>50</sub> μM)†		
	1 h	3 h	6 h	COX-1	COX-2	COX-2 selectivity Index‡
Vehicle	0.50 ± 0.07	1.76 ± 0.12	1.90 ± 0.12	–	–	–
Celecoxib	0.26 ± 0.07 (48%)	0.38 ± 0.10 (78%)	1.29 ± 0.13 (32%)	15.27 ± 1.6	0.43 ± 0.1	35.51
<b>5a</b>	0.38 ± 0.03 (24%)	1.12 ± 0.19 (36%)	1.54 ± 0.19 (19%)	16.35 ± 2.3	12.35 ± 1.6	1.32
<b>5b</b>	0.31 ± 0.02 (38%)	1.19 ± 0.11 (32%)	1.37 ± 0.12 (30%)	>30	10.58 ± 2.2	>2.83
<b>5c</b>	0.31 ± 0.03 (38%)	1.15 ± 0.12 (35%)	1.29 ± 0.12 (35%)	>30	11.02 ± 1.9	>2.72
<b>5d</b>	0.19 ± 0.02 (62%)	0.67 ± 0.12 (62%)	0.88 ± 0.14 (54%)	19.24 ± 3.1	1.53 ± 2.4	12.58
<b>6a</b>	0.47 ± 0.05 (6%)	1.54 ± 0.13 (13%)	1.79 ± 0.12 (6%)	14.68 ± 2.1	7.58 ± 1.2	1.94
<b>6b</b>	0.19 ± 0.02 (62%)	0.48 ± 0.12 (73%)	0.58 ± 0.09 (69%)	16.35 ± 1.9	4.68 ± 1.1	3.49
<b>6c</b>	0.44 ± 0.03 (12%)	1.58 ± 0.02 (10%)	1.69 ± 0.15 (9%)	>30	8.25 ± 1.6	>3.64
<b>6d</b>	0.18 ± 0.02 (64%)	0.47 ± 0.12 (73%)	0.50 ± 0.03 (74%)	17.57 ± 1.8	0.93 ± 1.3	18.79

\*Values are means ± SEM (n = 4–5).

†Values are expressed as mean ± SEM of three determinations (n = 3).

‡Selectivity index (COX-1 IC<sub>50</sub>/COX-2 IC<sub>50</sub>).

Table 3. Analgesic effect of test compounds **5a–d**, **6a–d** compared to piroxicam on thermal pain induced by hot-plate in mice.

Treatment	Latency (s)*		
	Basal	1 h (% change)	2 h (% change)
Saline	14.6 ± 3.2	13.2 ± 1.7	10.3 ± 3.1
Piroxicam	12.6 ± 2.3	24.5 ± 3.7 (94.44%) <sup>†</sup>	21.8 ± 4.5 (73.02%) <sup>†</sup>
<b>5a</b>	13.0 ± 1.9	14.3 ± 2.8 (10.00%)	13.9 ± 4.2 (6.92%)
<b>5b</b>	13.3 ± 2.3	14.9 ± 4.0 (12.03%)	11.7 ± 3.1 (13.65%)
<b>5c</b>	12.9 ± 4.1	14.3 ± 1.8 (10.85%)	14.9 ± 2.9 (15.50%)
<b>5d</b>	14.2 ± 2.2	19.2 ± 1.6 (35.21%) <sup>‡</sup>	14.7 ± 3.2 (3.52%)
<b>6a</b>	13.7 ± 3.2	14.2 ± 2.4 (3.65%)	12.6 ± 3.4 (8.73%)
<b>6b</b>	14.5 ± 2.9	18.9 ± 2.1 (30.34%) <sup>‡</sup>	17.4 ± 2.3 (20.00%) <sup>‡</sup>
<b>6c</b>	14.0 ± 3.1	18.7 ± 1.3 (33.57%) <sup>‡</sup>	18.3 ± 1.8 (30.71%) <sup>‡</sup>
<b>6d</b>	12.7 ± 3.0s	21.4 ± 3.1 (68.50%) <sup>‡</sup>	19.1 ± 2.6 (50.39%) <sup>‡</sup>

\*Values are means ± SD of four mice per group. Statistical comparisons between basal and post-drug values were analyzed for statistical significance using one-way ANOVA followed by Dunnett's test and denoted by <sup>‡</sup> $p < 0.05$  and <sup>†</sup> $p < 0.01$ .

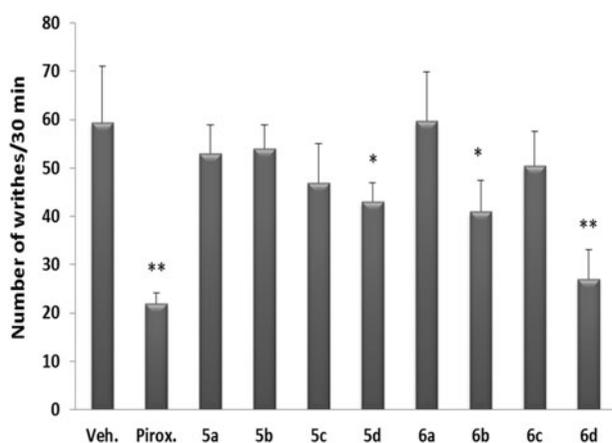


Figure 3. Analgesic effect of test compounds compared to piroxicam on acetic acid-induced writhing in mice. Test compounds were tested for their analgesic activity using the acetic acid-induced writhing test in mice. Piroxicam was used as a reference standard analgesic drug. Data represent the mean value ± SD of four mice per group. Statistical comparisons between basal and post-drug values were analyzed for statistical significance using one-way ANOVA followed by Dunnett's test and denoted by <sup>\*</sup> $p < 0.05$ , <sup>\*\*</sup> $p < 0.01$ .

compounds are clearly parallel to their anti-inflammatory activities.

#### Molecular modeling

Since the anti-inflammatory activity is one of the most important biological activities of the prepared compounds **5a–d**, **6a–d** and taking in consideration that cyclooxygenase-2 (COX-2) is the most relevant enzymatic system for inflammation<sup>11–14</sup>, a molecular modeling study was performed to understand the protein-inhibitor interactions and the structural features of the COX-2 active site and also, to investigate the possible binding conformation for the newly prepared compounds to this binding site, which may give a suggestion about their proposed mechanism of action as anti-inflammatory agents. Molecular docking study was done using the crystal structure of COX-2 (protein database [PDB] entry: 3NL1), with the prepared compounds and celecoxib as the reference ligand. The MOE version 2008.10 (Montreal, Quebec, Canada) modeling software (Molecular Operating Environment) was used for this study<sup>15</sup>. First of all, the celecoxib ligand was flexibly docked to the binding site, and the docking conformation corresponding to the lowest energy score value was

selected as the most probable binding conformation, then we docked the prepared compounds **5a–d** and **6a–d**. Docking studies showed that these compounds occupied the COX-2 binding site as compared with celecoxib (Figure 4).

Within the chalcone series **5a–d**, the binding energies for the most active derivatives **5d**, **5b** were found to be  $-18.71$ ,  $-10.42$  kcal/mol compared to  $-18.27$  kcal/mol observed for celecoxib. Furthermore, compounds **5d** and **5b** exhibited two and three hydrogen bonding interactions, respectively, in comparison to three hydrogen bonding interactions exhibited by celecoxib. Similarly, within the dihydropyrazole series **6a–d**, the most active compounds **6d**, **6b** showed the highest binding energy scores ( $-11.59$ ,  $-13.68$  kcal/mol, respectively) and the highest hydrogen bonding interactions (two for **6d** and three for **6b**) in comparison to celecoxib. The docking scores, amino acid interactions and the hydrogen bond lengths were summarized in Table 4.

#### Conclusion

In summary, two series of new compounds, 4'-flouro-2'-hydroxychalcones **5a–d** and the corresponding dihydropyrazoles **6a–d** were synthesized for biological evaluation as antioxidant, anti-inflammatory and analgesic activities. The antioxidant activity studies showed that the dimethoxychalcone **5a** and the corresponding dihydropyrazole **6a** exhibited the highest activities which support data obtained from literature.<sup>10</sup> While, the anti-inflammatory activity studies showed that monomethoxychalcone **5d** displayed good anti-inflammatory activity (54–62%), and the corresponding dihydropyrazoles **6d** showed excellent anti-inflammatory activity (64–74%) comparable to that of celecoxib (32–78%). In addition, the docking experiments showed that compounds **5d** and its cyclized derivative **6d** better fit into the COX-2 active site, which was in parallel correlation with the *in vitro* COX inhibition evaluation. Furthermore, the analgesic activities of these compounds are clearly parallel to their anti-inflammatory activities. The relatively potent anti-inflammatory and analgesic activities exhibited by chalcone **5d** and the corresponding dihydropyrazole derivative **6d** support the drug design concept that cyclization of chalcones to the corresponding dihydropyrazole analogues maintain or increase the anti-inflammatory and analgesic activities.

#### Experimental

##### Chemistry

Melting points were determined on a Griffin apparatus and are uncorrected. IR spectra were recorded on a Shimadzu 435 spectrometer using KBr discs. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were measured on a Bruker 400 MHz spectrometer (Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt) in D<sub>2</sub>O, DMSO-d<sub>6</sub> with tetramethylsilane as the internal standard, where *J* (coupling constant) values were estimated in hertz (Hz). Mass spectra were run on Hewlett Packard 5988 spectrometer (Palo Alto, CA). Microanalysis was performed for C, H, N at the (Micro Analytical Center, Cairo University, Egypt) and was within ± 0.4% of theoretical values. All other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification.

##### General method for preparation of 1,3-diarylprop-2-en-1-ones (**5a–d**)

4'-Flouro-2'-hydroxyacetophenone (**3**, 2.0 mmol, 0.38 g) and the appropriate substituted benzaldehyde (**4a**, **4b**, **4c** or **4d**, 2.0 mmol) were dissolved in a minimum volume of ethanol (5–10 mL). A 10% sodium hydroxide solution (4 mL) was added slowly and



7.95 (d,  $J = 8.7$  Hz, 1H, fluorophenyl H-6), 13.25 (s, 1H, OH, D<sub>2</sub>O exchangeable); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  15.1, 105.3, 107.2, 117.1, 118.6, 125.9, 129.1, 130.8, 131.9, 143.3, 145.2, 166.1, 168.7, 192.4; MS ( $m/z$ ): 288 (M<sup>+</sup>, 97%) 137 (100%); Anal Calcd for C<sub>16</sub>H<sub>13</sub>FO<sub>2</sub>S: C, 66.65; H, 4.54; found: C, 66.84; H, 4.92.

*1-(4-Fluoro-2-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (5d)*. Yield (82%); yellow crystals; mp 122–124 °C; IR (KBr disk) 3430 (OH), 3073 (C–H aromatic), 2966 (C–H aliphatic), 1641 (CO); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  3.88 (s, 3H, OCH<sub>3</sub>), 6.67–6.74 (m, 2H, fluorophenyl H-3, H-5), 6.99 (d,  $J = 8.7$  Hz, 2H, methoxyphenyl H-3, H-5), 7.37 (d,  $J = 8.7$  Hz, 2H, methoxyphenyl H-2, H-6), 7.56 (d,  $J = 15.6$  Hz, 1H, COCH = CH), 7.91 (d,  $J = 15.6$  Hz, 1H, COCH = CH), 7.94 (d,  $J = 8.7$  Hz, 1H, fluorophenyl H-6), 13.18 (s, 1H, OH, D<sub>2</sub>O exchangeable); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  55.4, 105.3, 107.3, 113.8, 116.7, 120.2, 121.3, 130.1, 135.8, 145.7, 160.0, 166.2, 168.8, 192.5; MS ( $m/z$ ): 272 (M<sup>+</sup>, 5%) 80 (100%); Anal. Calcd for C<sub>16</sub>H<sub>13</sub>FO<sub>3</sub>: C, 70.58; H, 4.81; found: C, 70.89; H, 4.49.

#### General method for preparation of 3,5-diaryl-4,5-dihydro-1H-pyrazoles (6a–d)

To a solution of the appropriate chalcone **5a**, **5b**, **5c** or **5d** (1.0 mmol) in ethanol (10 mL), hydrazine hydrate (1.2 mmol, 0.06 g) was added, and the reaction mixture was refluxed for 2–4 h. The reaction was monitored every 60 min interval on TLC plates using chloroform:methanol (9:1 V/V). The excess solvent was removed in vacuum, and the residue was washed with water, crystallized from absolute ethanol/methanol mixture (1:1) to give the respective dihydropyrazoles **6a–d** for which physical and spectral data are listed below.

*5-(2,3-Dimethoxyphenyl)-3-(4-fluoro-2-hydroxyphenyl)-4,5-dihydro-1H-pyrazole (6a)*. Yield (78%); white crystals; m.p. 96–98 °C; IR (KBr disk) 3427–3373 (OH, NH), 3079 (C–H aromatic), 2938 (C–H aliphatic), 1609 (C=N); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  3.09 (dd,  $J = 16.5, 8.7$  Hz, 1H, pyrazole H-4), 3.56 (dd,  $J = 16.5, 10.2$  Hz, 1H, pyrazole H'-4), 3.89 (s, 3H, OCH<sub>3</sub>), 3.90 (s, 3H, OCH<sub>3</sub>), 5.23 (ddd,  $J = 10.2, 8.7, 0.9$  Hz, 1H, pyrazole H-5), 6.60 (ddd,  $J = 7.8, 1.8, 0.9$  Hz, 1H, dimethoxyphenyl H-6), 6.72 (dd,  $J = 7.8, 1.8$  Hz, 1H, dimethoxyphenyl H-4), 6.91 (dd,  $J = 7.8, 7.8$  Hz, 1H, dimethoxyphenyl H-5), 7.04–7.27 (m, 3H, fluorophenyl H-3, H-5, H-6), 10.55 (s, 1H, NH, D<sub>2</sub>O exchangeable), 13.27 (s, 1H, OH, D<sub>2</sub>O exchangeable); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>)  $\delta$  40.9, 56.2, 56.7, 60.8, 103.5, 106.8, 112.5, 114.4, 118.0, 124.5, 129.7, 136.2, 146.7, 152.8, 159.0, 161.9, 164.3; MS ( $m/z$ ): 316 (M<sup>+</sup>, 0.2%) 271 (100%); Anal Calcd for C<sub>17</sub>H<sub>17</sub>FN<sub>2</sub>O<sub>3</sub>: C, 64.55; H, 5.42; N, 8.86; found: C, 64.69; H, 5.57; N, 8.86.

*5-(3,4-Dimethoxyphenyl)-3-(4-fluoro-2-hydroxyphenyl)-4,5-dihydro-1H-pyrazole (6b)*. Yield (69%); white crystals; m.p. 144–146 °C; IR (KBr disk) 3393–3331 (OH, NH), 3071 (C–H aromatic), 2934 (C–H aliphatic), 1605 (C=N); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  3.22 (dd,  $J = 6.5, 8.7$  Hz, 1H, pyrazole H-4), 3.61 (dd,  $J = 16.5, 10.2$  Hz, 1H, pyrazole H'-4), 3.87 (s, 3H, OCH<sub>3</sub>), 3.88 (s, 3H, OCH<sub>3</sub>), 4.97 (dd,  $J = 10.2, 8.7$  Hz, 1H, pyrazole H-5), 6.61 (dd,  $J = 8.4, 2.4$  Hz, 1H, dimethoxyphenyl H-2), 6.72 (dd,  $J = 8.4, 2.4$  Hz, 1H, dimethoxyphenyl H-6), 6.78 (d,  $J = 8.4$  Hz, 1H, dimethoxyphenyl H-5), 6.91–6.97 (m, 2H, fluorophenyl H-3, H-5), 7.15–7.27 (m, 1H, fluorophenyl H-6), 10.53 (s, 1H, NH, D<sub>2</sub>O exchangeable), 13.31 (s, 1H, OH, D<sub>2</sub>O exchangeable); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>)  $\delta$  41.4, 55.9, 56.0, 62.5, 103.6, 106.8, 111.0, 112.2, 114.4, 119.3, 129.8, 134.8, 149.2, 152.7, 159.1, 161.9, 164.3; MS ( $m/z$ ): 316 (M<sup>+</sup>, 35%) 151 (100%); Anal Calcd for C<sub>17</sub>H<sub>17</sub>FN<sub>2</sub>O<sub>3</sub>: C, 64.55; H, 5.42; N, 8.86; found: C, 64.82; H, 5.61; N, 9.11.

*3-(4-Fluoro-2-hydroxyphenyl)-5-(4-methylsulfanylphenyl)-4,5-dihydro-1H-pyrazole (6c)*. Yield (65%); pale yellow crystals; m.p.

152–154 °C; IR (KBr disk) 3408–3324 (OH, NH), 3066 (C–H aromatic), 2917 (C–H aliphatic), 1602 (C=N); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  2.47 (s, 3H, SCH<sub>3</sub>), 3.29 (dd,  $J = 16.5, 8.7$  Hz, 1H, pyrazole H-4), 3.65 (dd,  $J = 16.5, 10.2$  Hz, 1H, pyrazole H'-4), 5.01 (dd,  $J = 10.2, 8.7$  Hz, 1H, pyrazole H-5), 6.64–6.80 (m, 3H, fluorophenyl H-3, H-5, H-6), 7.13–7.33 (m, 4H, methylsulfanylphenyl H-2, H-3, H-5, H-6), 10.52 (s, 1H, NH, D<sub>2</sub>O exchangeable), 13.25 (s, 1H, OH, D<sub>2</sub>O exchangeable); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>)  $\delta$  15.7, 40.4, 60.3, 103.5, 106.9, 113.9, 126.3, 127.2, 132.1, 134.6, 138.1, 155.7, 161.3, 165.1; MS ( $m/z$ ): 302 (M<sup>+</sup>, 0.12%) 80 (100%); Anal Calcd for C<sub>16</sub>H<sub>15</sub>FN<sub>2</sub>OS: C, 63.56; H, 5.00; N, 9.26; found: C, 63.19; H, 5.03; N, 9.39.

*3-(4-Fluoro-2-hydroxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole (6d)*. Yield (70%); yellow crystals; m.p. 115–117 °C; IR (KBr disk) 3409–3321 (OH, NH), 3056 (C–H aromatic), 2932 (C–H aliphatic), 1609 (C=N); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  3.30 (dd,  $J = 16.5, 8.7$  Hz, 1H, pyrazole H-4), 3.67 (dd,  $J = 16.5, 10.2$  Hz, 1H, pyrazole H'-4), 3.93 (s, 3H, OCH<sub>3</sub>), 5.13 (dd,  $J = 10.2, 8.7$  Hz, 1H, pyrazole H-5), 6.67–6.86 (m, 3H, fluorophenyl H-3, H-5, H-6), 7.18–7.48 (m, 4H, methylsulfanylphenyl H-2, H-3, H-5, H-6), 10.55 (s, 1H, NH, D<sub>2</sub>O exchangeable), 13.31 (s, 1H, OH, D<sub>2</sub>O exchangeable); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>)  $\delta$  40.3, 55.8, 60.9, 103.8, 106.6, 111.5, 113.4, 117.3, 128.2, 135.1, 152.7, 159.6, 162.6, 165.0; MS ( $m/z$ ): 286 (M<sup>+</sup>, 0.03%) 80 (100%); Anal Calcd for C<sub>16</sub>H<sub>15</sub>FN<sub>2</sub>O<sub>2</sub>: C, 67.12; H, 5.28; N, 9.78; found: C, 66.87; H, 5.17; N, 9.95.

#### Biological evaluation

##### Animals

Swiss albino mice 20–25 g and Wistar rats (150–175 g) were obtained from the National Research Center, Cairo, Egypt) were used throughout the study and were kept at controlled conditions (temperature 23 ± 2 °C, humidity 60 ± 10%) and a 12/12 h light/dark cycle. All procedures relating to animal care and treatments were conducted in accordance with protocols approved by the Research Ethical Committee of Faculty of Pharmacy Beni-Suef University (2014-Beni-Suef, Egypt).

##### Material

DPPH radical was obtained from Sigma-Aldrich (St. Louis, MO). Ascorbic was purchased from Merck (Rahway, NJ). All the used chemicals and solvents were of analytical grade.

##### DPPH radical scavenging activity

Antioxidant activity was evaluated as the scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical essentially as mentioned before<sup>16–18</sup>. In a brief, 2 mL of DPPH solution (0.2 mmol/L in ethanol) was incubated with different concentrations of the test compounds. The reaction mixtures were shaken and wrapped in aluminum foil and kept at room temperature for 30 min in dark. The spectrophotometric measurements at 517 nm were done under dim light. All data are presented as mean ± standard deviation (SD) ( $n = 3$ ). The IC<sub>50</sub> (the concentration of sample necessary to cause 50% inhibition of DPPH radical scavenging activity) was calculated for each compound. Ascorbate (Vitamin C) was used as a positive antioxidant control.

##### Yeast-based antioxidant screening assay

The yeast-based biological assay detects antioxidant activities of test compounds against physiologically relevant oxidants. DPPH radical scavenging activity method provides only an indication of the ability of a compound to scavenge oxidants<sup>19</sup>. For this reason,

we used yeast-based method, which can also measure the ability of a compound to induce cellular resistance to the damaging effects of oxidants<sup>19</sup>. Antioxidant activity screening was done in a 96-well microplate high throughput assay using *Saccharomyces cerevisiae* as described before<sup>18,20</sup>. Briefly, *S. cerevisiae* (BY4743) were cultured overnight then diluted to an optical density of 0.2 at 600 nm (OD600) in the media. Diluted culture (180  $\mu$ L) was added per well in a 96-well plate, and then 10  $\mu$ L of test compound was added to a final concentration of 8 mM. H<sub>2</sub>O<sub>2</sub> (10  $\mu$ L) was added into each well to a final concentration of 4 mM. The OD600 reading as an indicator of yeast growth was taken using a microplate reader before and after the incubation at 30 °C with shaking for 20 h. Ascorbic acid was used as a positive control. The net growth of yeast after the treatment with H<sub>2</sub>O<sub>2</sub> and test compounds was measured using the following equation:

$$P_{\text{Yeast growth}} = \frac{(P_{\text{Sample}} - P_{\text{Control}})}{P_{\text{Control}}} \times 100$$

where  $P_{\text{Yeast growth}}$  = net growth of yeast cells after treatment with test compounds;  $P_{\text{Sample}}$  = observed optical density of yeast cells with the treatment with test compounds;  $P_{\text{Control}}$  = observed optical density of yeast cells with the treatment of negative control.

#### COX-1/COX-2 inhibition colorimetric assay

The *in vitro* inhibition of COX-1/COX-2 was measured using colorimetric COX (ovine) Inhibitor Screening Assay Kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions and as mentioned before<sup>21</sup>. Different concentrations of test compounds or celecoxib were incubated with the enzymes for 5 min at 25 °C. After the incubation and addition of colorimetric substrate and arachidonic acid, the absorbance was measured at 590 nm using plate reader.

#### Carrageenan-induced rat paw edema assay

Rats were administered vehicle, test compounds (10 mg/kg) or Celecoxib (10 mg/kg) by oral gavage. Immediately thereafter and under light anesthesia, the animals received 100  $\mu$ L of vehicle or carrageenan (1% in saline) s.c. on the plantar surface of the left hindpaw as reported before<sup>22,23</sup>. The development of paw edema was assessed by measuring paw-volume changes at 1, 3 and 6 h after carrageenan injection using plethysmometer. The right hindpaw served as a reference of non-inflamed paw for comparison. Results are expressed as paw-volume change (ml).

#### Hot-plate test

The test was performed as mentioned before<sup>24</sup>. Basal, 1 and 2 h values of hot-plate latency (seconds) of saline (control) and compounds treated mice (10 mg/kg). Piroxicam (10 mg/kg) was used as positive control. Compounds were administered by oral gavage 1 h before testing. A cutoff latency of 30 s was used to prevent heat-induced tissue damage.

#### Acetic acid-induced writhing test

The writhing tests were carried as described before<sup>25</sup>. Briefly, mice were pre-treated orally by vehicle, test compounds (10 mg/kg) or piroxicam (10 mg/kg) 30 min prior to intraperitoneal administration of 0.7% v/v acetic acid solution at 10 ml/kg body weight. The number of writhes (i.e. abdominal constriction followed by dorsiflexion and extension) occurring during a 30-min period beginning 5 min after acetic acid injection

was measured. The results are expressed as the number of writhes per 30-min period.

#### Statistical analysis

Comparisons among groups were analyzed for statistical significance using one-way ANOVA followed by Dunnett's test. Data were represented as mean  $\pm$  SD. Differences were considered significant at \* $p < 0.05$ , and \*\* $p < 0.01$ , respectively.

#### Molecular modeling and docking

The crystal structure of celecoxib bound at the *Mus musculus* COX-2 active site (obtained from protein data bank at research collaboration for structural Bioinformatics PDB entry 3LN1) was used in this study<sup>15</sup>. Docking of the co-crystallized ligand should be carried out to study the scoring energy(s), root mean standard deviation and amino acid interactions. The root mean square deviation, which is measure of superposing, was 0.50 Å for the lead compound. Docking was performed using London dG force, and refinement of the results was done using Force field energy.

Preparation of the synthesized compounds for docking was achieved *via* their 3D structure built by MOE. Certain procedures should be taken before docking, which include: 3D protonation of the structures, running conformational analysis using systemic search, selecting the least energetic conformer and applying the same docking protocol used with ligand.

The previous measures were taken and docking for the synthesized compounds was applied. Amino acid interactions and the hydrogen bond lengths were summarized in Table 4.

#### Declaration of interest

The authors have declared no conflict of interest.

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